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American Academy of Allergy, Eleventh Annual Meeting, Statler Hotel, New York City, New York, February 7, 8, 9, 1955

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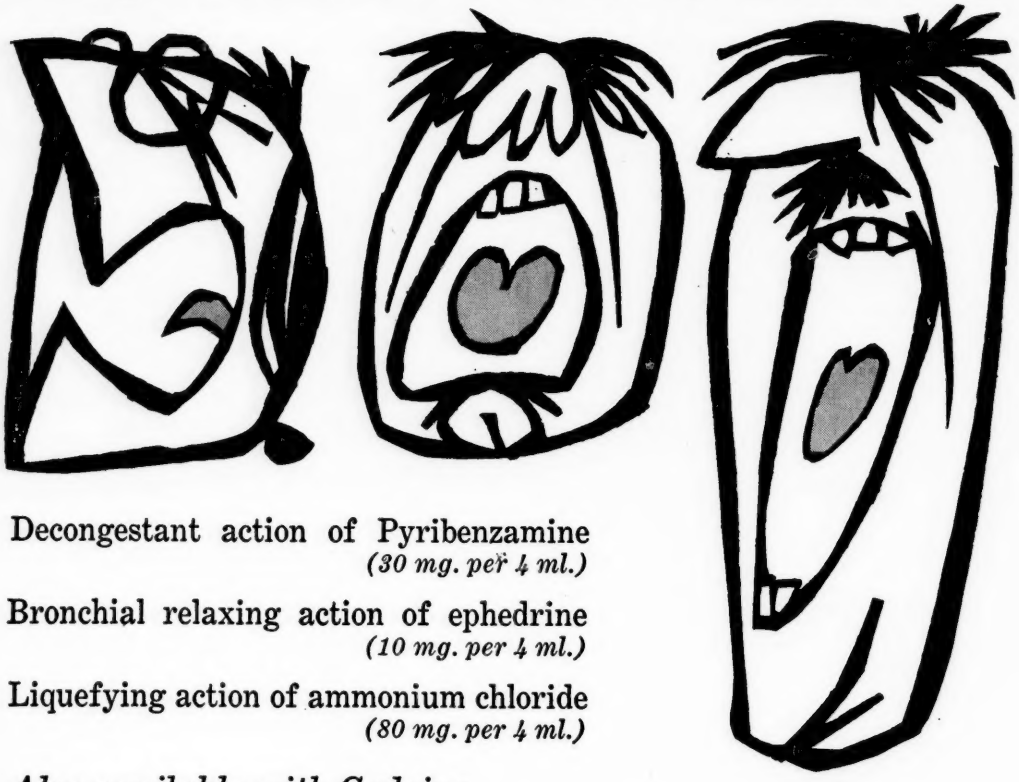

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The Journal of Allergy

VOL. 26 JANUARY, 1955 No. 1

Original Articles

CORTICOTROPHIN, CORTISONE, AND HYDROCORTISONE IN DISEASES OF HYPERSENSITIVITY

I. BIOLOGICAL CORTICOID EXCRETION DURING ACUTE SYMPTOMS

B. ROSE, M.D., T. W. FYLES, M.D.,* AND E. H. VENNING, PH.D.,
MONTREAL, QUEBEC

INTRODUCTION

THE use of corticotrophin in many forms of allergy has become widespread following the initial demonstration of the efficacy of these compounds in asthma.[1, 2, 3] Since corticotrophin stimulates the adrenal cortex to release a variety of steroids, including cortisone and hydrocortisone, it becomes necessary to know whether there is a relative pituitary or adrenal insufficiency in asthma or whether the allergic patient is incapable of responding to the stress of his disease with an increase in adrenal activity. In preliminary reports, Rose and associates[4] showed that during acute asthma there was no indication of a pituitary-adrenal response and, in addition, that the average output of adrenocortical steroids was lower in asthma patients than in normal individuals. The present report is a further amplification of these initial findings.

METHODS

The patients, with few exceptions, were asthmatics whose symptoms were persistent and difficult to control by routine measures. All urines were collected during active asthma for which routine therapy, other than cortisone or corticotrophin, was given when necessary. Comparison of results was based on groups divided as to sex, type of asthma (intrinsic or extrinsic), and ease of control.

The urinary glucocorticoids were assayed by the method of Venning, Kazmin, and Bell.[5] This assay is based on the determination of compound E-like substances by biologic assay. The results obtained by this method are expressed in glycogen units; the activity of one glycogen unit is equivalent to the

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*Medical Research Fellow, National Research Council, Canada, 1953 to 1954.

activity of one gamma of cortisone. Urinary creatinine was assayed on each forty-eight-hour urine sample and corticoid results discarded if the creatinuria showed the sample to be incomplete. In one patient the urinary corticoids were estimated by a chemical method.[6]

RESULTS

1. *The Effect of Bed Rest on the Corticoid Excretion in Asthma.*—In a single case of the extrinsic type, a man aged 29, with asthma since childhood, the urinary corticoids were estimated over a period of a week during which the symptoms abated. No therapy other than bed rest and mild sedation was given.

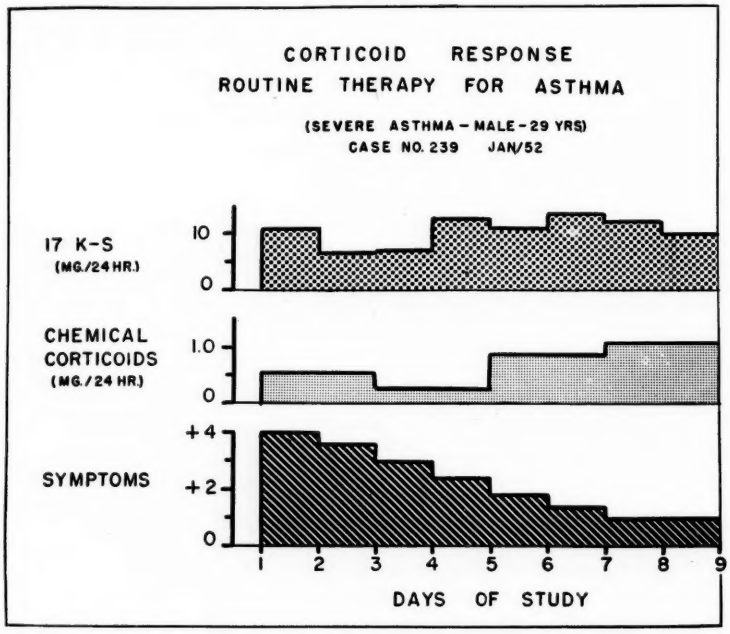


Fig. 1.—The urinary corticoid (chemical) and 17-ketosteroid response in a young asthmatic patient who made a spontaneous recovery during hospitalization. No hormone therapy was administered. The rise in chemical corticoids accompanied by a decrease in the symptoms will be seen.

As will be seen in Fig. 1, there was a significant increase in the level of the chemical corticoid excretion, the values rising from an initial value of 0.5 to an ultimate value of 1.0 mg. per twenty-four hours. This type of response was in contradistinction to that seen in the asthmatic patient whose symptoms persisted despite hospitalization and symptomatic treatment, and whose values remained low until the administration of corticotrophin or cortisone. An example of this is shown in Fig. 2 and is similar to that shown in previous publications.[4]

2. *The Effect of Nonspecific Therapy on Urinary Corticoid Excretion.*—It is well known that an acute infection or some other form of acute stress will often suppress an existing attack of asthma and will also stimulate the adrenal

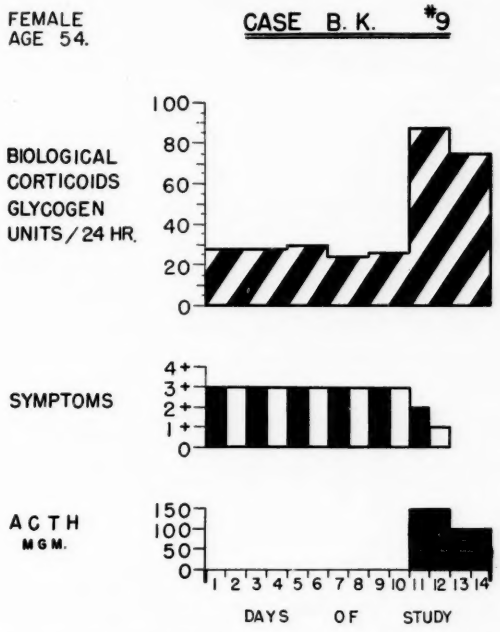
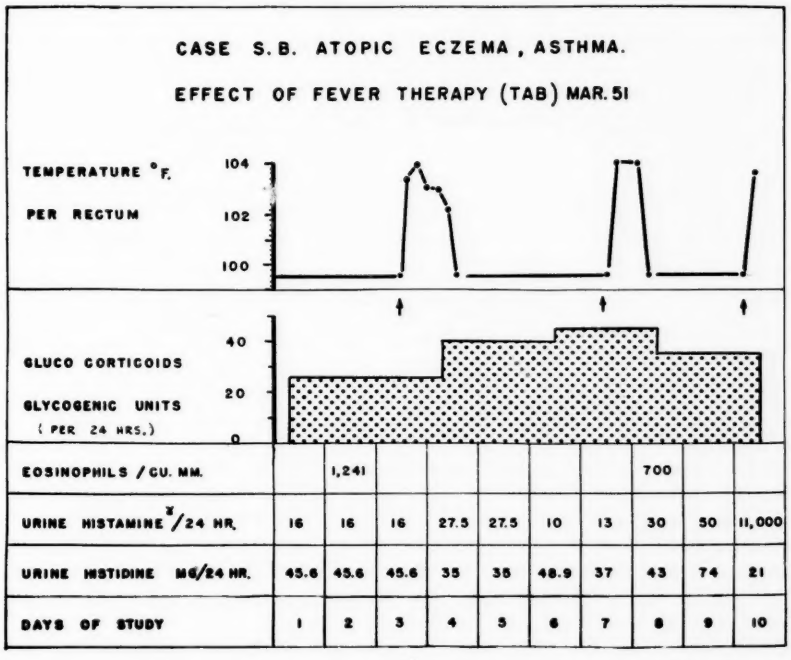


Fig. 2.—The urinary biologic corticoid response in an older asthmatic patient whose symptoms persisted while hospitalized until corticotrophin was administered. The biologic corticoid excretion increased with hormone therapy and the symptoms decreased.



EOSINOPHILS / CU. MM.		1,241						700		
URINE HISTAMINE γ/24 HR.	16	16	16	27.5	27.5	10	13	30	50	11,000
URINE HISTIDINE MG/24 HR.	45.6	45.6	45.6	35	35	48.9	37	43	74	21
DAYS OF STUDY	1	2	3	4	5	6	7	8	9	10

Fig. 3.—The urinary biologic corticoid response in a young asthmatic patient who received nonspecific fever therapy. The symptoms improved with treatment and the rise in the corticoid excretion is illustrated. Injections are indicated by the arrows.

cortex to release large quantities of glucocorticoids in the urine.[7] For this reason, the urinary glucocorticoids were assayed in a patient with active asthma and atopic dermatitis before and during fever therapy. The results are shown in Fig. 3. It will be seen that following the induction of fever by means of intravenous typhoid-paratyphoid vaccine there occurred a significant increase in the urinary glucocorticoid excretion within twenty-four hours. Associated with this increased output of glucocorticoids, there was a drop in the eosinophils and improvement in the symptoms. This is another example of alleviation of symptoms associated with augmentation of pituitary-adrenal activity by a nonspecific stimulus.

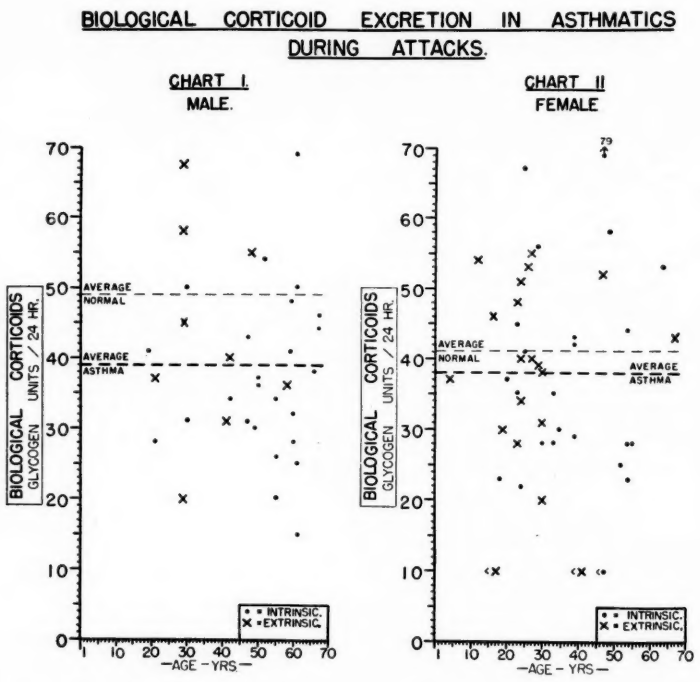


Fig. 4.—The biologic corticoid excretion in male and female asthmatics during attacks. The values for patients with intrinsic asthma are represented by a dot and those for patients with extrinsic asthma by a cross. The average excretion of the male asthmatics is considerably lower than the normal male average, but the average excretion of the female asthmatics is only slightly lower than the normal female average.

3. *Urinary Corticoid Excretion During Attacks of Asthma.*—In Fig. 4 are shown the scattergrams of seventy-eight estimations of biologic corticoids on a group of fifty-eight patients divided according to sex. As previously noted, these assays were carried out during active asthma and are charted according to age. The average normal excretion values by this method, according to Venning[8] are: men, 49 (range 32 to 75); women, 41 (range 25 to 65). As can be seen, the average value for men is 39, whereas that for women is 38 glycogen units per twenty-four hours for this group of subjects. It will be

noted that the average values in these groups are lower than those of normal subjects and that many of the estimations fall below the lower limit of the normal range.

In a further attempt to analyze these findings, the patients were divided into those with intrinsic asthma, indicated by the dots, and those with extrinsic asthma, indicated by the crosses in Fig. 4. It will be noted that there was no significant difference between the values obtained for these groups divided on this basis.

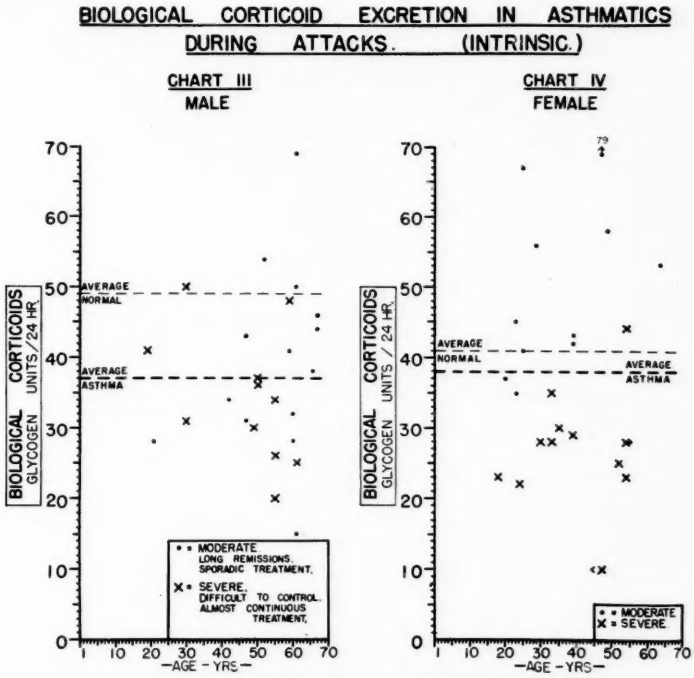


Fig. 5.—The biologic corticoid excretion in male and female patients with intrinsic asthma during attacks. The values for patients with moderate asthma are represented by a dot and those for patients with severe asthma by a cross. The average excretion of the male intrinsic asthmatics is considerably lower than the normal male average, but the average excretion of the female intrinsic asthmatics is only slightly lower than the normal female average.

In Fig. 5 are shown the values obtained in thirty-six patients with intrinsic asthma. There were twenty-five estimations in seventeen male and twenty-four estimations in nineteen female patients. As can be observed, the average value for the men was 37 and for the women 38 glycogen units per twenty-four hours. The findings are similar to those found in the previous analysis. A further attempt to analyze the results on these same patients was made by subdividing them on the basis of clinical evaluation, response to therapy, and ease of management; those difficult to control are signified by a cross and those readily controlled are indicated by a dot (Fig. 5). It appears that those in the latter category have a higher level of steroid excretion than do those whose symptoms were severe and difficult to treat. This is more

marked for the women (average: moderate, 49; severe, 27 glycogen units per twenty-four hours) than for the male patients (average: moderate, 39; severe, 34) in this group.

A smaller group comprising six male and sixteen female patients with extrinsic asthma on whom nine estimations were made in the former and twenty in the latter are shown in Fig. 6. The average value for the men was 43 and for the women 38 glycogen units per twenty-four hours. These patients were again divided into severe, represented by a cross, and moderate as shown by a dot.

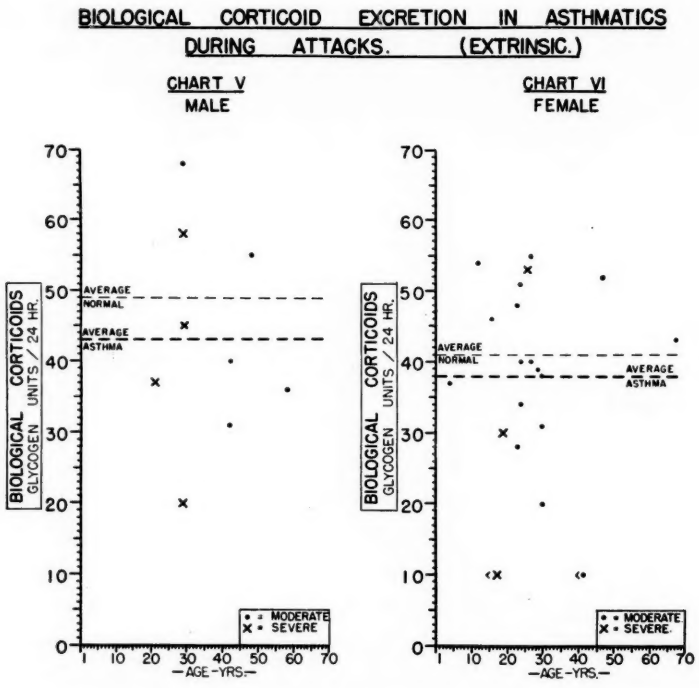


Fig. 6.—The biologic corticoid excretion in male and female patients with extrinsic asthma during attacks. The values for patients with moderate asthma are represented by a dot and those for patients with severe asthma by a cross. The average excretion of both the male and female extrinsic asthmatics is lower than their respective normal averages.

The number of assays does not permit of any definite conclusions, although it is interesting to note that in both male and female groups the severe cases showed a lower average excretion than did the moderate cases (male average: moderate—46, severe—40 glycogen units per twenty-four hours; female average: moderate—39, severe—31 glycogen units per twenty-four hours).

4. *Urinary Corticoid Excretion in Urticaria and Atopic Dermatitis.*—Two cases of urticaria and nine of atopic dermatitis are charted in Fig. 7. It will be seen that in no instance does the level of corticoid excretion fall below the range of normal, contrary to the findings in asthmatic patients. It is also interesting that in the two cases of urticaria, therapy with corticotrophin had no effect on the symptoms.

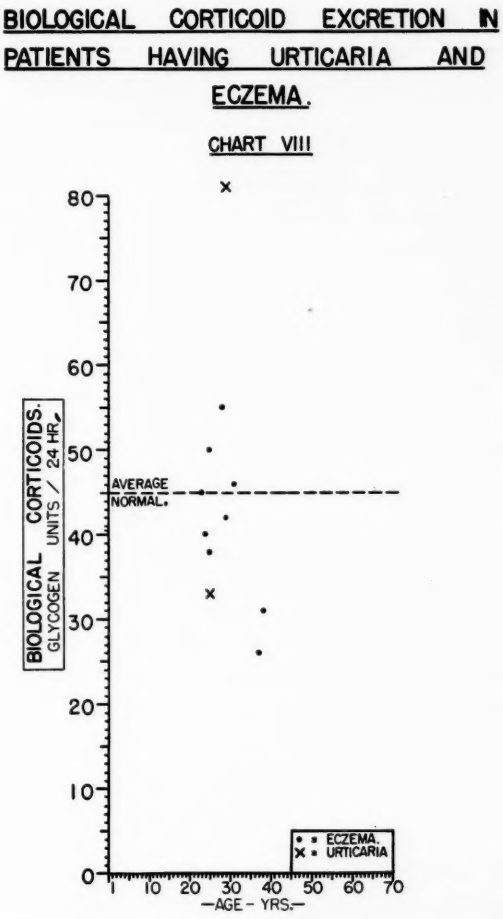


Fig. 7.—The biologic corticoid excretion in male and female patients with urticaria and eczema. The patients with eczema are represented by a dot and those with urticaria by a cross. It will be seen that the values obtained in these cases are grouped about the average normal excretion value.

DISCUSSION

Although the response of the adrenal cortex to trauma, infections, burns, and other acute states is thought to be of a nonspecific nature and is associated with a marked increase of corticoid excretion in the urine, there are many basic factors associated with hypersensitivity which seem to be under the direct influence of the pituitary-adrenal mechanism. For example, adrenalectomy in animals leads to an increase in the ease of sensitization and in the severity of anaphylactic symptoms.[9-11] In early investigations on histamine, which is responsible for many of the major symptoms in classic anaphylactic shock, a marked increase of this amine was shown to exist in tissues[12] and the capacity of these tissues to inactivate histamine was grossly impaired in the adrenalectomized animal.[13] This was thought to be due to a decrease in the activity of histaminase, the enzyme specific for histamine.[14] This finding has

recently been confirmed by Kahlson and associates.[15] Another important factor in the hypersensitive state is antibody formation. Although antibody production is not uniformly altered, it is well established that a considerable increase occurs during sensitization in adrenalectomized rabbits as compared with intact animals. This increase is associated with a greater severity of symptoms or specific lesions such as periarteritis nodosa.[9, 10, 16, 17] There are other facets of the hypersensitive state which are altered in some way by adrenalectomy, but these are considered in detail elsewhere.[18]

In man there are many observations relating to histamine metabolism which establish that it differs in the allergic patient as compared with the nonallergic individual. For example, in previous studies Rose showed that the blood histamine remains stable in the normal individual whereas it varies markedly in patients with asthma or atopic dermatitis.[19] In addition, it was noted that asthmatics tend to excrete relatively higher amounts of histamine in the urine than do normal individuals.[20] During pregnancy the enzyme histaminase rises in the plasma. Asthma during pregnancy tends to be associated with a decreased histaminase activity.[21] In some instances a lowered adrenocortical activity in the urine as measured by biologic corticoid excretion studies is found.[22] Recently Parrot[23] has shown that the blood plasma of normal individuals has the capacity to bind histamine without destroying it. This activity is not present in the blood of asthmatic patients or in the blood of adrenalectomized guinea pigs, but is found to be present in the blood of intact animals.

It was on the basis of previous observations relating to the effects of adrenalectomy on histamine metabolism that ACTH was first used in therapeutic trials for asthma, preliminary reports of which were published a number of years ago.[2, 20] Our basic interest, therefore, was to establish whether there might be some relation between adrenocortical activity and the asthmatic state, in view of the well-established therapeutic effect of ACTH.

The interpretation of urinary glycogenic corticoid excretion must be approached with some caution. It is known from studies in Addison's disease and Cushing's syndrome that the corticoid excretion in the urine is a reliable index of adrenocortical activity.[24] It is likely that the relationship holds for other diseases as well, but this has not been as clearly shown. The administration of therapeutic doses of corticotrophin and cortisone in the presence of an intact adrenal gland further complicates this problem. With these reservations in mind, the findings herein reported are of considerable interest.

There are many determinations in this series which indicate that the activity of the adrenal cortex is unusually low in bronchial asthma as compared with average values for normal subjects. The reason for this low excretion of biologic corticoids is not readily apparent. It may be a reflection of the decreased adrenocortical activity found in many chronic debilitating diseases[8] and may be partly responsible for the continuation of asthmatic symptoms. It is probable that spontaneous recovery from asthma is associated with an increase of adrenocortical activity as shown in Fig. 1. The

degree of stimulation, however, as reflected by this bio-assay method is much less than is observed following severe trauma, surgical operation, or burns. It is well known that an acute infection, such as pneumonia or measles, or a surgical operation will usually terminate severe asthma, with a disappearance of acute symptoms and a drop in the eosinophilia if such is present. This must certainly be due to stimulation of the pituitary-adrenal mechanism as shown by the studies of Browne and associates.[7] It is felt, therefore, that the onset of asthma or the allergic state may occur either in the presence of a normally functioning adrenal cortex or, in some instances, a hypofunctioning adrenal. There may be several reasons for the persistence of symptoms in relation to pituitary-adrenal activity. It is possible that the stimulus resulting from the asthmatic state is in itself insufficient to stimulate pituitary activity. Presuming it to be adequate, it may be blocked in some way. One other unlikely possibility is that the pituitary-adrenal mechanism is incapable of responding to this form of stimulus. In the extrinsic forms of allergy there can be no doubt that persistence of symptoms is due in large measure to the presence of the particular antigen involved. This may not be the only factor involved, however, since many such patients retain positive skin tests but lose their clinical susceptibility without specific treatment. This may be related in some way to the pituitary-adrenal mechanism.

It seems clear in the case of man, at least, that the degree of severity of existing asthma is in some way related to adrenocortical activity and the ability of the adrenal to respond. It appears to be equally clear, however, that the tendency to become allergic is in no way related to adrenocortical activity. For example, the incidence of allergy in Addison's disease is apparently no greater than is found in the normal population.[25]

SUMMARY

The excretion of urinary glucocorticoids was studied in a group of fifty-eight patients during active asthma. It was shown that persistence of symptoms is not associated with a rise in the level of glucocorticoid excretion in the urine. Spontaneous recovery from asthma and recovery induced by nonspecific therapy, however, are associated with a rise in urinary corticoid excretion. The results suggest that the asthmatic patient may excrete smaller quantities of urinary corticoids than do normal subjects and that the persistence of symptoms may be due in part to an inability of the pituitary or adrenal to augment the output of corticoids or to respond to the stimulus of asthma. The results in two cases of urticaria and nine of atopic dermatitis do not differ significantly from those found in normal subjects, and in this respect they differ from the asthmatic patients.

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THE EFFECT OF HELIUM-OXYGEN MIXTURES ON PULMONARY FUNCTION IN ASTHMATIC PATIENTS

IRVING W. SCHILLER, M.D., FRANCIS C. LOWELL, M.D., MARY T. LYNCH, M.D., AND WILLIAM FRANKLIN, M.D., BOSTON, MASS.

BECAUSE helium and oxygen mixtures have been recommended for the relief of severe bronchial asthma,[1, 2] we undertook a study of the influence of this mixture on pulmonary function in this disease. Studies in dogs[3] and in healthy human subjects[4] suggest that such mixtures are advantageous under conditions of high rates of flow, and also where there is localized obstruction in the upper airway.[5] Under the circumstances just mentioned, turbulence is considered to be a factor in resistance to gas flow. In streamline flow, however, which occurs in narrowed passages, viscosity of the gas becomes the major factor in resistance to flow; and helium has a viscosity 10 per cent greater than that of nitrogen. Changes within the lung, other than resistance to airflow, may contribute to respiratory difficulty in patients with pulmonary disease and these are unaffected by the physical characteristics of the gas being breathed. For example, hydrogen, a gas with low density and viscosity, failed to prevent the increased work required to move air in and out of the lung, induced in the experimental animal by pilocarpine.[6]

METHODS

The patient seated and rested, and with a noseclip and earpiece of a Millikan oximeter in place, breathed through a flanged rubber mouthpiece connected to a low resistance two-way valve assembly with a dead space of approximately 60 c.c.; gas was supplied from one of two reservoirs connected with a spirometer recording on two kymographs; the first was run continuously at low speed in order to obtain a spirogram in the usual manner; the second intermittently at higher speed in order to obtain records suitable for measurement of velocity of airflow. The two reservoirs, having a capacity of approximately 120 liters each, contained, respectively, room air and a mixture composed of 80 per cent helium and 20 per cent oxygen. The helium mixture, freshly drawn from a tank, was cooler than the air and this difference was appreciated by some of the patients. The arrangement was such that the expired air could not mix with that to be inspired, obviating the need for a CO_2 scrubber. Three-way valves connecting the two gas reservoirs permitted abrupt change from one gas reservoir to the other. This apparatus has been described in more detail elsewhere.[7]

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Measurements of the expiratory reserve volume, the inspiratory capacity, the vital capacity, and the volume expired in the first second during the performance of the vital capacity were made at intervals of two minutes or more. No measurement was made until the patient breathed air or the helium mixture for at least six minutes, and in patients with severe disease this period was extended.

Breathing gas from one reservoir, the patient was instructed to make a maximal expiratory effort (expiratory reserve volume). The patient then breathed quietly for two minutes or more and the measurement was repeated. These were followed by two vital capacities performed in a similar manner. During these measurements the high-speed kymograph was set in motion in order to obtain tracings suitable for measurement of the speed of airflow. The gas mixture was changed to 80 per cent helium and 20 per cent oxygen and the measurements were again carried out as described. The largest measurement obtained with each gas mixture was selected and these constituted a pair. In some patients the entire procedure was carried out two or three times in the course of a morning or afternoon. Unfortunately, patients with very severe asthma tolerated these tests poorly, and in these it was not always possible to obtain all the afore-mentioned measurements. In some, the procedure had to be abandoned. As a result, values obtained from the less sick patients tended to predominate in this study.

RESULTS

In all, a total of thirty-seven pairs of values was obtained in eight patients. Three patients were tested on two occasions. The paired results were plotted as shown in Fig. 1, the value for air being measured along the abscissa and that for the helium mixture along the ordinate. Were the values obtained with the helium mixture no different from those obtained with air, the points for all the paired values would have fallen on the straight line running diagonally upward from the origin.

No difference was evident between the results obtained with air and the helium mixture in the expiratory reserve volume. There likewise was no significant difference in the inspiratory capacity, although a very slight trend in favor of helium is evident in three values over 2,500 c.c. Among eight paired measurements of the volume expired in the first second, there was no significant difference in six. In two, the values were 25 per cent and 30 per cent greater with the helium mixture. Both of these were in subjects who expired more than 1,200 c.c. in the first second. Seven paired measurements of the vital capacity showed no difference when these were less than 2,900 c.c.; among five pairs in which the value was greater than 2,900 c.c., three favored the helium mixture and one favored air.

Using the oximeter, the blood oxygen saturation was followed in most of our patients. In no instance did we observe any significant change in oxygen saturation when the subjects who were breathing air were given the helium mixture for six to ten minutes.

Measurements of the resaturation rate[8] proved too protracted for sick patients, and this procedure was omitted in all but the initial studies. No conclusion could be drawn from these.

The minute volume was increased in most patients upon the administration of the helium mixture, and this increase occurred in the apparent absence of increased effort on the part of the patient. One patient who was relatively well at the time the tests were made, maintained that breathing was easier.

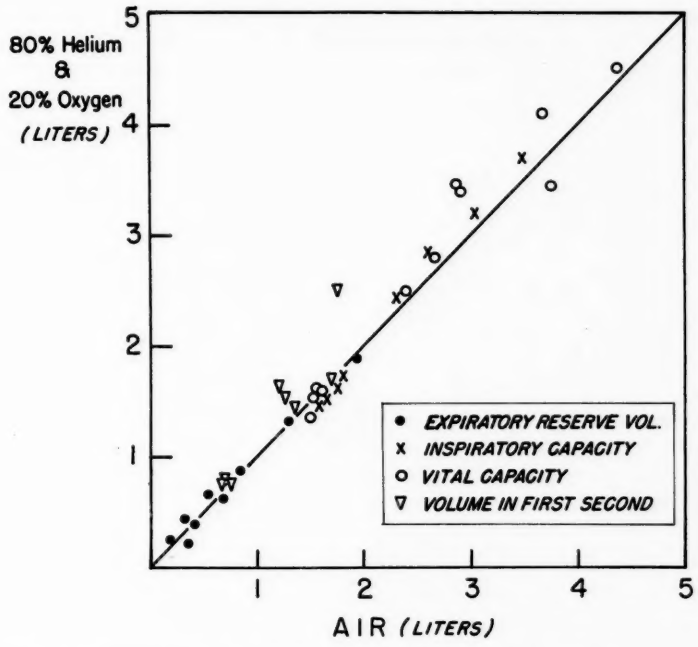

Fig. 1.—Comparison of ventilatory measurements made in asthmatic patients with air and a mixture containing 80 per cent helium and 20 per cent oxygen. Each point represents two measurements. Those measurements made with air are plotted along the abscissa, those with the helium-oxygen mixture along the ordinate.

After tests with air and the helium mixture had been completed, each patient was given six inhalations of isopropylarterenol, 1:200, from a No. 40 DeVilbiss nebulizer, and measurements of the vital capacity and the expiratory rate were repeated. In each instance, as was to be expected, a significant increase in these measurements occurred and always exceeded any change obtained with the helium-oxygen mixture.

DISCUSSION

These studies fail to show any clear-cut difference between air and a mixture of 80 per cent helium and 20 per cent oxygen. Some isolated pairs of values favor the helium mixture, but these were observed only when the volume of gas expired was relatively large. In other words, in the sicker patients the helium mixture appeared to have no advantage over air. Although too sick to tolerate these measurements, one patient developed severe respiratory

difficulty while breathing air from the apparatus and, after obtaining some relief from aerosolized isopropylarterenol, also developed severe asthma after breathing the helium mixture for about ten minutes, so that the administration of this gas had to be interrupted and medication again given.

The theoretical advantage that a helium and oxygen mixture may have with respect to the diffusion of oxygen within the lung spaces might have been reasonably expected to be reflected by an increase in the blood oxygen saturation as measured by the oximeter. This failed to occur, even though the minute volume increased in the majority of patients upon breathing the helium mixture.

Alternative explanations for the increase in minute volume observed with the helium mixture are: (1) decreased resistance to flow in the external circuit (tubing, valves, and spirometer), or (2) decreased resistance to the initiation of flow at the beginning of inspiration, resulting in a tendency to "overshoot" with each breath. These, however, do not explain the absence of a rise in arterial saturation upon breathing the helium mixture.

If helium mixtures, which differ from air chiefly in density, are indeed of no value in patients with severe asthma, then turbulence would not appear to be a significant factor in the dyspnea of asthma. On the other hand, streamline flow and, therefore, the viscosity of the gas mixture, would appear to play a major role. The part played by tissue viscance in bronchial asthma remains to be studied. Observations in this laboratory made in asthmatic subjects on a rapidly moving kymograph have shown consistently that, in the performance of the vital capacity, the asthmatic patient can inspire rapidly even if expiration is slow. This point is readily seen in published spirograms obtained in patients with emphysema.[10] Such tracings, obtained in both asthmatic and emphysematous patients, indicate that if tissue viscance plays an important role in slowing the airflow, then the unlikely supposition must be made that the opposing force to deformation presented by the lung tissue is different in magnitude, depending on whether the lung is expanding or contracting. In other words, tissue viscance would be expected to vary with the phase of respiration. Recently, evidence was obtained with a body plethysmograph[9] in patients with pulmonary emphysema that obstruction of the airway, rather than tissue viscance, was the factor which accounted for the slow rate of airflow in such patients.

The two factors, density and viscosity, may conceivably influence the manner in which the inspired gas is distributed within the lung depending on the patency of the bronchial tree in different parts of the lung. If we assume that viscosity is the most important property governing gas flow within the asthmatic lung, then the possibility presents itself that a helium mixure might be disadvantageous. The helium mixture with a viscosity no less than that of air would not be expected to pass more readily through the partially obstructed, narrowed airways because of the role played by viscosity under such circumstances. Therefore, better ventilation of the poorly ventilated spaces in the lung is not to be expected. If there are portions of the asthmatic lung where rapid flow through large passages occurs, and where

turbulence is a factor in resistance to flow, then these portions of the lung, already well-ventilated, would become better ventilated if a less dense gas is given. This would be of little advantage in the circumstances under consideration because blood perfusing such areas is already well saturated and can acquire very little additional oxygen. If the well-ventilated areas of the lung become better ventilated (when a low density gas is breathed) without a change in minute volume, this must occur at the expense of the ventilation of the poorly ventilated spaces, and the over-all effectiveness of the respiratory effort will be diminished. In other words, in order to maintain the ventilation of poorly ventilated portions of the lung, the minute volume, especially the tidal volume, would have to be increased. This may explain the apparent paradox that although an increase in minute volume was seen in most instances upon the administration of the helium mixture, there was no concomitant increase in the arterial oxygen saturation as measured by oximeter.

SUMMARY

Eight patients with bronchial asthma were given air and a helium-oxygen mixture to breathe, and spirograms and measurements of speed of flow were obtained. In the severely ill patients, no significant change was observed in the expiratory reserve volume, the inspiratory capacity, the vital capacity, or the speed of flow during the performance of the vital capacity. In some measurements among the less ill patients, slightly larger values were obtained with helium.

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THE PREPARATION OF HIGHLY CONCENTRATED ALLERGENIC EXTRACTS BY INCREASING THE CONCENTRATION OF EXTRACTION

I. Timothy and Low Ragweed Pollens

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EARLIER impressions concerning pollen extraction[4, 16] implied that it was not advantageous to extract pollen at concentrations greater than 3 per cent to 8 per cent weight by volume. This opinion was based on the observation that the relative activity of such extracts apparently failed to increase in proportion to the increase in extracting concentration.

Brown, in 1932,[4] reported on yields of extraction up to 20 per cent weight by volume ratio. The more concentrated extracts, as a group, were more active by direct skin tests than the less concentrated ones. The total nitrogen values increased in regular proportion up to 12.5 per cent, but at 17.5 per cent and 20 per cent they were not proportionately stronger. Stull and his associates,[42] in 1933, briefly reported studies using a maximum weight by volume ratio of 9 per cent. The values of total and phosphotungstic acid-precipitable nitrogen of extracts, ranging between 1 per cent and 9 per cent concentrations, were linear even after the volume of the filtrates was adjusted to the original volume of fluid added. Sullivan and Vaughan[46] found that a 10 per cent extract was five times more active than a 2 per cent extract. Rappaport[36] prepared an extract which was four and one-half times stronger than the original extract by drying at 40° C. and restoring to the desired volume. There are reports of the use of 10 per cent to 20 per cent extracts for clinical and experimental purposes.[6, 16, 24, 30, 35, 37, 47] Malkiel[27] first informed this laboratory of his findings that the yields of nitrogen increased beyond that to be expected from an examination of the early reports on this subject.

Considerable advantage could result from the preparation of pollen extracts of higher concentration, since those prepared in the proportion of 3 to 5 Gm. per 100 ml. extracting fluid, have been found to be relatively poor antigens for lower animal experimentation. It was felt that greater concentrations of extracts would offer more desirable antigens, not only for sensitization of the lower animal, but also for clinical use.

Furthermore, since it has been shown that glycerin in the strength of 50 per cent preserves the activity of pollen extracts[20] and since the injection of

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such high concentrations of glycerin may be painful, it seemed desirable to prepare stronger extracts, so that when dilutions of such extracts are prepared for clinical use the glycerin content would be of weaker, more practical strengths.

The present investigation was designed to determine the effect of increasing the weight by volume ratios on the concentration of allergenic extracts.

METHODS

Preparation of Extracts.—Extracts of low ragweed (*Ambrosia elatior*) and timothy (*Phleum pratense*) pollens were prepared by adding weighed aliquots (0.5 to 25 grams) of a single lot of ether-defatted (Soxhlet) pollen to 50 ml. aliquots of extracting fluid. Evans-buffered saline[17] was designated as "plain" extracting fluid and Evans-buffered saline containing glycerin in the concentration of 50 per cent was designated as "glycerinated" extracting fluid. Fifty per cent glycerin was used, since this concentration of glycerin had been shown to maintain stability of ragweed pollen extracts.[20]

Direct extraction was carried out with 50 per cent glycerinated Evans fluid. It was evident that this procedure would preclude the dilution that necessarily results when glycerin is added to stock solutions after extraction. There are many reports of the use of glycerin as an extractant.[1-3, 5, 7, 40,46]

The weight by volume ratios of pollen to extracting fluid used in this study were 5, 10, 20, 30, 40, and 50 grams per 100 ml. These extracts were designated as 5, 10, 20, 30, 40, and 50 per cent extracts, respectively. All extracts were processed in an identical manner. Thorough mixing and wetting were accomplished by manual agitation. The pollen suspensions were then placed in an automatic shaker for one hour at 4° C. and refrigerated overnight. The insoluble residue was removed by Buchner filtration through a single sheet of Whatman #1 filter paper and the volume of each filtrate measured. Discrepancies due to the effect of Seitz filtration were minimized by passing a standard volume (20 ml.) through the sterilizing filter pad (St-1, 37 mm.). The Seitz filtrates were dispensed into sterile containers, cultured, and stored at 4° C.

Analytic Procedures.—The analytic techniques which were used in these studies were essentially as follows:

Nitrogen content was determined by micro-Kjeldahl methods described by Conway,[9] Ma and Zuazaga,[26] and Miller and Houghton.[29]

Phosphotungstic acid-precipitable nitrogen was determined according to the method of Cooke and Stull.[10] Hereafter this value will be referred to as PTA nitrogen. One protein nitrogen unit (PNU) is equivalent to 0.00001 mg. phosphotungstic acid-precipitable nitrogen.

Reducing substances, hexoses, and pentoses, were quantitatively estimated by the methods of Somogyi,[38] Nelson,[32] Meybaum,[28] and Lusena.[25] Hydrolysis was carried out with 5 N sulfuric acid for three hours at approximately 100° C., followed by neutralization with sodium hydroxide. All values of concen-

trations of reducing substances were reported as glucose in milligram per milliliter. The values of bound carbohydrate were obtained by subtracting the values of free reducing substances in unhydrolyzed samples from their corresponding values of total reducing substances (after hydrolysis).

Qualitative chromatograms for identification of amino acid and carbohydrate components of the substances analyzed were run according to the methods of Consden and co-workers,[8] Dent,[15] and Partridge.[33, 34] Hydrolysis for amino acid chromatograms was accomplished with 6 N hydrochloric acid in sealed tube at 110° C. for eighteen hours followed by drying and aeration over sodium hydroxide. Hydrolysis for carbohydrate chromatograms was accomplished with 1 N sulfuric acid for one hour at 100° C. followed by neutralization with barium hydroxide.

Determinations of pH were performed with a Beckmann research G apparatus.

The allergenic activity of each extract was estimated by the quantitative determination of its capacity to neutralize skin-sensitizing antibody (reagin), according to the method of Cooke and co-workers,[11, 12] using pooled high-titer serum obtained from patients with ragweed and timothy pollinosis. Briefly, the technique was as follows: Equal volumes of a single dilution of serum capable of passively sensitizing normal human skin to ragweed and timothy pollens were mixed with the same volume of a series of dilutions of each extract. Two to six hours after in vitro mixture, 0.1 ml. of each mixture was injected intradermally into the backs of nonreactive human subjects. Ninety-six hours later, each site was tested with approximately 0.02 ml. of a known active extract containing 1,000 phosphotungstic acid-precipitable nitrogen units (PNU) per milliliter. The immediate wheal reactions were evaluated after about thirty minutes. The lowest concentration of extract present in the serum—extract mixture which inhibited a positive reaction on subsequent retest was designated as the end point of neutralization.

RESULTS

Physical Characteristics of Extracts.—Freshly prepared extracts showed certain differences as the concentration of extracting mixtures was increased from 5 to 50 per cent weight by volume.

The volume of the Buchner filtrate decreased from 96 to 44 per cent of the original volume of fluid added for extraction. The time required for Seitz filtration increased approximately sixfold. The pH of the filtrates decreased from 6.0 to 5.4. The clarity of the Buchner filtrates decreased. The color intensity increased.

Several changes in appearance of the extracts were noted after varying periods of storage at refrigerator temperature.

Sedimentation occurred in all unglycerinated extracts of both timothy and ragweed pollens. There was increased sedimentation with increasing per cent extraction. Crystals appeared in certain extracts of timothy. Observations on the nature of these deposits are described in detail in the *Sedimentation* section of this article.

The pH of undiluted, one-year-old extracts showed a drop from their original values (Table I). The plain extracts, as a group, tended to show greater fall than the glycerinated extracts. The greatest change appeared in the plain 40 per cent weight by volume ragweed extract, which showed a drop from 5.62 to 4.93. The range of pH of one-year-old extracts was 5.95 (5 per cent) to 4.9 (40 per cent), compared with the original range of 6.0 to 5.4.

TABLE I. DETERMINATION OF pH OF UNDILUTED EXTRACTS

	PER CENT EXTRACTION (WEIGHT BY VOLUME)	ORIGINAL	pH 1 YEAR AT 4° C.	CHANGE
Ragweed Pollen				
Plain	5	6.00	5.95	−0.05
	10	5.82	5.50	−0.32
	20	5.70	5.18	−0.52
	40	5.62	4.93	−0.69
50% Glycerinated	5	5.92	5.80	−0.12
	10	5.70	5.75	+0.05
	20	5.63	5.20	−0.33
	30	5.59	5.25	−0.24
	40	5.58	5.20	−0.38
Timothy Pollen				
Plain	5	6.00	5.60	−0.40
	10	5.70	5.28	−0.42
	20	5.50	5.20	−0.30
	40	5.40	5.12	−0.28
50% Glycerinated	5	6.00	5.85	−0.15
	10	5.82	5.56	−0.26
	20	5.62	5.51	−0.11
	30	5.60	5.51	−0.09
	40	5.60	5.43	−0.17
	50	5.60	5.42	−0.18
Extracting Fluids				
Plain and 50% Glycerinated		7.00	7.00	0.00

Dilution of the extracts with buffered saline to concentrations of 0.10 to 0.001 mg. PTA nitrogen per milliliter, in some instances, resulted in a slight increase in pH after storage for one year. Criep and associates[13] noted a similar change in pH with aging of pollen extracts that had been diluted with buffered saline to concentrations of 0.01 mg. nitrogen per milliliter.

The color of most of the extracts became darker with age, particularly the plain extracts of high weight by volume concentration.

Chemical Analyses.—

A. Total and phosphotungstic acid-precipitable nitrogen: There was a linear, proportional increase in the total and PTA nitrogen content of both plain and glycerinated ragweed and timothy pollen extracts as the weight by volume ratio of extraction was increased from 5 to 50 per cent (Table II). These results have been confirmed by the analysis of extracts, prepared in a similar manner, of four other lots of timothy and ragweed pollens.

The total and PTA nitrogen concentrations of the fresh extracts, when plotted against concentrations of extraction, yielded straight lines (Figs. 1 and 2). No plateau was encountered with the extraction concentrations used in this study with either timothy or ragweed pollen.

TABLE II. ONE-DAY-OLD POLLEN EXTRACTS*; ANALYTICAL DATA

		WEIGHT BY VOLUME RATIO							
		5 PER CENT	10 PER CENT	20 PER CENT	30 PER CENT	40 PER CENT	50 PER CENT	AVERAGE	K-VALUE** (RANGE)
Ragweed									
Plain	PTA-N	0.18	0.42	0.79	----	1.92	----	0.041	(0.036-0.048)
	TOTAL-N	0.54	1.00	2.04	----	4.33	----	0.105	(0.100-0.108)
	BOUND-CHO	2.30	4.60	9.00	----	18.50	----	0.460	(0.450-0.460)
	TOTAL-CHO	4.70	9.06	18.00	----	36.00	----	0.911	(0.900-0.911)
50% Glycerinated	PTA-N	0.16	0.36	0.75	1.20	1.74	----	0.038	(0.032-0.044)
	TOTAL-N	0.41	0.83	1.62	2.60	3.59	----	0.085	(0.081-0.090)
	BOUND-CHO	1.85	4.26	8.45	12.60	16.90	----	0.412	(0.370-0.430)
	TOTAL-CHO	3.70	8.50	17.00	25.00	34.40	----	0.826	(0.740-0.860)
Timothy									
Plain	PTA-N	0.27	0.52	1.14	----	2.45	----	0.055	(0.052-0.061)
	TOTAL-N	0.46	0.83	1.95	----	4.15	----	0.094	(0.083-0.104)
	BOUND-CHO	***							
	TOTAL-CHO	13.00	25.00	48.00	----	95.00	----	2.470	(2.400-2.600)
50% Glycerinated	PTA-N	0.23	0.48	1.06	1.58	2.16	2.65	0.051	(0.046-0.054)
	TOTAL-N	0.35	0.70	1.55	2.55	3.10	3.65	0.075	(0.070-0.085)
	BOUND-CHO	***							
	TOTAL-CHO	12.00	23.50	46.00	65.00	90.00	110.00	2.280	(2.190-2.400)

PTA–N = Phosphotungstic acid-precipitable nitrogen (milligram per milliliter).
TOTAL–N = Total nitrogen (milligram per milliliter).
BOUND–CHO = Total reducing substances minus free reducing substances (as glucose; milligram per milliliter).
TOTAL–CHO = Total reducing substances (hydrolyzed samples) (as glucose; milligram per milliliter).
*Seitz-filtered extracts.
**K-value = Ratio of concentration in milligram per milliliter to per cent extraction.
***Timothy extracts showed no increase, often a slight decrease, in CHO upon hydrolysis.

The total recovery of PTA nitrogen was progressively less, even though the concentration of PTA nitrogen increased with increasing concentration of extraction. Table III tabulates the relative recoveries, obtained with ragweed extracts in the upper half and timothy extracts in the lower half. The extraction ratio for both glycerinated and plain extracts is shown in the first column. There was progressive reduction in volume recovered as the concentration of extraction increased, until with glycerinated 40 per cent ragweed extract, for example, only 58 per cent of the added volume of fluid was recovered as extract, as recorded in the second column. Column 3 shows the linear increase of PTA nitrogen per milliliter of extract as the concentration increased. Column 4 expresses this increased protein content as per cent of the content of the 5 per cent weight by volume extract. Thus, the 40 per cent ragweed extract was 910 per cent stronger than the 5 per cent extract. Column 5 shows that the total PTA nitrogen recovered per 100 Gm. of extracted pollen decreased progressively as the concentration of extraction increased. This is

expressed in Column 6 as diminishing per cent of recovery. Therefore, the total yield of PTA nitrogen from glycerinated 40 per cent ragweed extract was only 72 per cent of that recoverable from the 5 per cent extract.

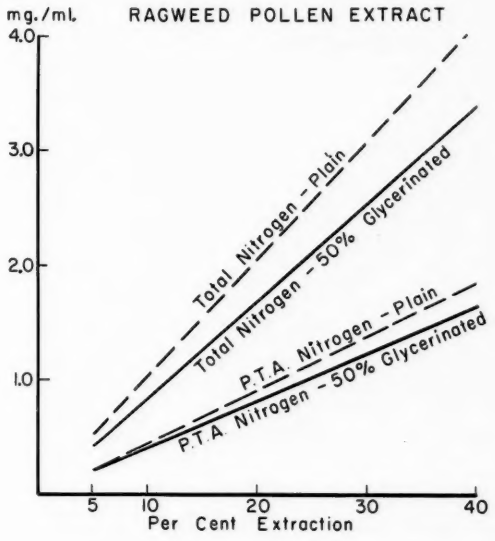

Fig. 1.

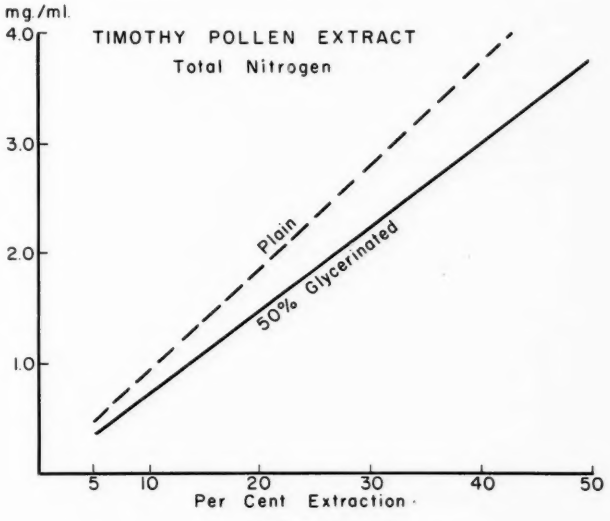

Fig. 2.

The same pattern of recovery was encountered for all the other extracts as recorded in Table III.

The total and PTA nitrogen values of glycerinated extracts of both timothy and ragweed pollen were 10 to 20 per cent lower than those of plain extracts (Tables II and III and Figs. 1, 2, and 3). Even though glycerin thus

TABLE III. RELATIVE RECOVERIES COMPARED WITH 5 PER CENT POLLEN EXTRACTS*

	PER CENT EXTRACTION (W/V)	PER CENT ORIGINAL VOLUME (50 ML.) RECOVERED	PTA NITROGEN (MG. PER ML.)	PER CENT OF 5% EXTRACT	TOTAL PTA-NITROGEN RECOVERED PER 100 GM. OF POLLEN (MG.)	PER CENT OF 5% EXTRACT
	(1)	(2)	(3)	(4)	(5)	(6)
Ragweed						
	5	92	0.20	100	368	100
	10	83	0.42	208	348	95
50% Glycerinated	20	68	0.84	420	286	78
	30	68	1.23	615	279	76
	40	58	1.82	910	264	72
	5	96	0.26	100	516	100
	10	92	0.48	178	440	85
Plain	20	82	0.95	356	391	76
	40	70	2.26	843	290	76
Timothy						
	5	94	0.28	100	533	100
	10	88	0.59	206	514	97
50% *Glycerinated*	20	74	1.18	416	436	82
	30	58	1.88	663	363	68
	40	46	2.59	913	298	56
	50	50	3.65	1.285	350	65
	5	90	0.35	100	624	100
	10	86	0.66	192	570	92
Plain	20	68	1.47	424	500	80
	40	44	3.13	905	345	55

*Fresh extracts, not Seitz-filtered.

TABLE IV. PHOSPHOTUNGSTIC ACID-PRECIPITABLE NITROGEN

	PER CENT EXTRACTION (W/V)	ORIGINAL	PTA-N. (MG. PER ML.) ONE YEAR AT 4° C.	PER CENT LOSS
Ragweed Pollen				
	5	0.18	0.15	17
Plain	10	0.42	0.30	29
	20	0.79	0.62	22
	40	1.92	1.68	13
	5	0.16	0.14	13
50% Glycerinated	10	0.36	0.33	8
	20	0.75	0.73	3
	30	1.20	1.12	7
	40	1.74	1.68	4
Timothy Pollen				
	5	0.27	0.16	41
Plain	10	0.52	0.30	43
	20	1.14	0.64	39
	40	2.45	1.38	44
	5	0.23	0.20	12
50% Glycerinated	10	0.48	0.45	6
	20	1.06	0.96	10
	30	1.58	1.48	6
	40	2.16	2.10	3
	50	2.65	2.58	3

reduced the yield of nitrogen, its use had the advantage of providing clear extracts in contrast to the extremely turbid and opalescent extracts obtained with plain extracting fluids, as described later in this article under *Sedimentation*.

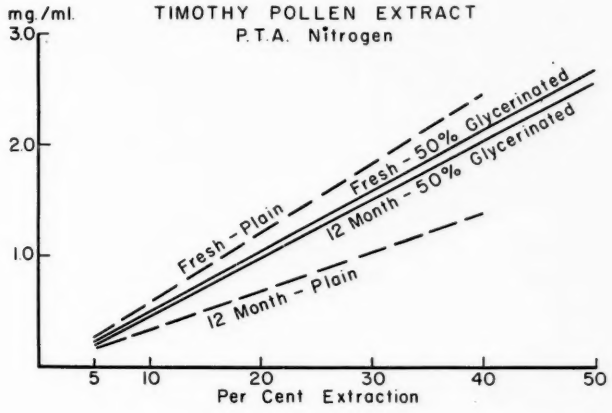

Fig. 3.

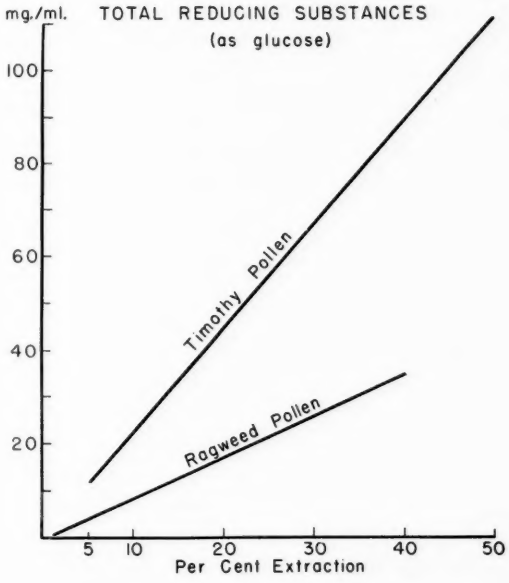

Fig. 4.

All extracts showed some loss of PTA nitrogen, varying from 2.5 to 44 per cent after storage for one year at 4° C. (Table IV and Fig. 3). The glycerinated extracts showed smaller losses than the unglycerinated extracts. This preservative effect of glycerin against loss of PTA nitrogen has been noted in other studies from this laboratory.[20]

B. Reducing substances: There was distinct linearity between concentration of extraction and content of reducing substances in extracts of both pollens (Table II and Fig. 4).

1. Ragweed Pollen Extracts:

All fresh extracts of ragweed pollen showed an increase of approximately 100 per cent in reducing substances after hydrolysis as shown by the values of bound and total carbohydrate in Table II.

Bound carbohydrate could no longer be detected in the plain ragweed extracts after storage for one year at 4° C., whereas the glycerinated extracts showed only very slight losses (Table V).

TABLE V. RAGWEED POLLEN EXTRACTS; BOUND REDUCING SUBSTANCES

PER CENT EXTRACTION (W/V)		MG. PER ML. (AS GLUCOSE) ORIGINAL	ONE YEAR AT 4° C.	PER CENT LOSS
Plain	5	2.30	0.30	87
	10	4.60	0.70	85
	20	9.00	0.80	91
	40	18.50	2.00	90
50% Glycerinated	5	1.85	1.80	3
	10	4.26	3.90	9
	20	8.45	8.10	4
	30	12.60	11.00	12
	40	16.90	14.00	17

Chromatography studies indicated that glucose and fructose were present before hydrolysis and glucose and arabinose after hydrolysis. These results are in agreement with those on ragweed extracts reported by Dankner and his co-workers[14] and Frankel and associates.[18]

2. Timothy Pollen Extracts:

The concentration of reducing substances in timothy pollen extracts showed no change, or only very slight losses, upon hydrolysis. Stull and his co-workers[41, 43] reported similar findings in studies with extracts of cattail and timothy pollens.

Aging did not result in any quantitative change in carbohydrate content, since all the measurable reducing substances in the timothy extracts were in the free form in contrast to those of the ragweed extracts.

Chromatographic studies of timothy extracts, both before and after hydrolysis, revealed the presence of glucose and fructose. Gough,[19] using methods other than chromatography, found l- arabinose and galactose after hydrolysis of sodium hydroxide extracts of timothy grass pollen. This apparently resulted in extraction of bound reducing substances and destruction of free reducing substances. This finding is in contrast to the studies on aqueous and buffered saline extracts of timothy in this study and that by Stull and associates[41, 43] in which quantitative determinations revealed no bound carbohydrate.

Calculation of the total recovery of reducing substances in relation to concentration of extraction yielded the same differences already described for the yields of PTA nitrogen.

Reagin Neutralization Studies.—The final criterion of allergenic yield should be evaluated in terms of biologic activity of the extracts, rather than any single chemical determination. In these studies, the allergenic activity of each extract was estimated by the quantitative determination of its capacity to neutralize skin-sensitizing antibody (reagin).

TABLE VI. REAGIN NEUTRALIZATION ACTIVITY OF NINETEEN-WEEK-OLD RAGWEED POLLEN EXTRACTS

CONCENTRATION OF EXTRACT MIXED WITH SERUM DILUTION (IN EQUAL VOLUMES) PNU* PER ML.	WEIGHT BY VOLUME EXTRACTION RATIO (PER CENT)							
	50% GLYCERINATED				PLAIN			
	5	10	20	40	5	10	20	40
5	+++	+++	++	+	+++	+++	+++	++
10	++	+	+	0	++	+++	+++	+
25	+	+	+	0	+	++	+	+
50	0	0	0	0	±	±	±	±
100	0	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0
800	0	0	0	0	0	0	0	0
Diluent	++++	++++	++++	++++	++++	++++	++++	++++

*PNU = Phosphotungstic acid-precipitable nitrogen units.

A. Ragweed pollen extracts: Six neutralization experiments were performed with relatively fresh ragweed extract. Five, 10, 20, and 40 per cent extracts, studied as described under *Methods,* after six, ten, and nineteen weeks' storage showed clear-cut linearity in that the neutralization points for each extract, when diluted to the same PTA nitrogen concentration, were essentially of the same values (Table VI). Earlier studies, when the extracts were three and four weeks old, showed an unexplained trend toward increasing activity with increasing per cent extraction (Table VII), which was encountered in three separate experiments.

TABLE VII. REAGIN NEUTRALIZATION ACTIVITY OF FOUR-WEEK-OLD RAGWEED POLLEN EXTRACTS

CONCENTRATION OF EXTRACT MIXED WITH SERUM DILUTION (IN EQUAL VOLUMES) PNU* PER ML.	WEIGHT BY VOLUME EXTRACTION RATIO (PER CENT)							
	50% GLYCERINATED				PLAIN			
	5	10	20	40	5	10	20	40
10	+++	+++	+++	++	+++	+++	+++	++
25	++	++	+	±	++	++	+	±
50	++	+	±	0	++	++	±	0
75	++	+	0	0	++	++	0	0
100	++	0	0	0	++	++	0	0
200	++	0	0	0	++	++	0	0
400	±	0	0	0	++	++	0	0
800	0	0	0	0	+	0	0	0
Diluent	++++	++++	++++	++++	++++	++++	++++	++++

*PNU = Phosphotungstic acid-precipitable nitrogen units.

Furthermore, each extract, after storage for one year at 4° C., neutralized human ragweed-sensitive serum in the same linear range (Table VIII). Incidentally, plain extracts, after storage for one year, showed a loss in potency in comparison with glycerinated extracts in that only 50 to 100 units of glycerinated, but as much as 100 to 400 units of the plain, extracts were required for neutralization of the reaginic serum used in this study (Table VIII). These findings are similar to those previously reported from this laboratory.[20] The results with plain extracts agree with those of Stull and Sherman[45] who noted a loss of PTA nitrogen and neutralization capacity of extracts after storage for ten months at 7° C.

TABLE VIII. REAGIN NEUTRALIZATION ACTIVITY OF ONE-YEAR-OLD RAGWEED POLLEN EXTRACTS

CONCENTRATION OF EXTRACT MIXED WITH SERUM DILUTION (IN EQUAL VOLUMES) (PNU* PER ML.)	WEIGHT BY VOLUME EXTRACTION RATIO (PER CENT)								
	50% GLYCERINATED					PLAIN			
	5	10	20	30	40	5	10	20	40
5	++++	++++	++++	+++	+++	++++	++++	++++	++++
10	++++	++++	+++	+++	+++–	++++	++++	++++	++++
25	++	++	++	++	+	+++	+++	+++	+++
50	0	+	±	+	0	+++	+++	+++	+++
100	0	0	0	0	0	+	+	+	+
200	0	0	0	0	0	+	0	0	±
400	0	0	0	0	0	0	0	0	0
800	0	0	0	0	0	0	0	0	0
Diluent	++++	++++	++++	++++	++++	++++	++++	++++	++++

*PNU = Phosphotungstic acid-precipitable nitrogen units.

B. Timothy pollen extracts: Three neutralization experiments, performed with four- and five-week-old timothy extracts revealed distinct uniformity of neutralization activity of all concentrations from 5 to 50 per cent weight by volume (Table IX). The plain extracts showed some loss in activity, after storage for one year, when compared with the glycerinated extracts (Table X), indicating the preservative effect of glycerin, as was encountered with ragweed extracts previously noted.

TABLE IX. REAGIN NEUTRALIZATION ACTIVITY OF FOUR-WEEK-OLD TIMOTHY POLLEN EXTRACTS

CONCENTRATION OF EXTRACT MIXED WITH SERUM DILUTION (IN EQUAL VOLUMES) (PNU* PER ML.)	WEIGHT BY VOLUME EXTRACTION RATIO (PER CENT)								
	50% GLYCERINATED					PLAIN			
	5	10	20	40	50	5	10	20	40
10	+++	+++	+++	+++	+++	+++	+++	+++	+++
50	±	±	0	±	±	±	±	0	0
100	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0	0
Diluent	++++	++++	++++	++++	++++	++++	++++	++++	++++

*PNU = Phosphotungstic acid-precipitable nitrogen units.

TABLE X. REAGIN NEUTRALIZATION ACTIVITY OF ONE-YEAR-OLD TIMOTHY POLLEN EXTRACTS

CONCENTRATION OF EXTRACT MIXED WITH SERUM DILUTION (IN EQUAL VOLUMES) (PNU* PER ML.)	WEIGHT BY VOLUME EXTRACTION RATIO (PER CENT)									
	50% GLYCERINATED						PLAIN			
	5	10	20	30	40	50	5	10	20	40
5	+	+	+	+	+	+	+	+	+	++
10	0	0	0	0	0	0	+	±	+	+
25	0	0	0	0	0	0	0	0	±	+
50	0	0	0	0	0	0	0	0	0	+
100	0	0	0	0	0	0	0	0	0	+
200	0	0	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0	0	0
Diluent	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

*PNU = Phosphotungstic acid-precipitable nitrogen units.

Table XI shows that, since the neutralization activity of each extract was linear, dilution of each fresh timothy extract to the same PTA nitrogen content resulted in approximately equal values when expressed in terms of total nitrogen content or of weight by volume dilutions.

TABLE XI. TIMOTHY POLLEN EXTRACTS; COMPARATIVE VALUES

	PER CENT EXTRACTION	PTA NITROGEN (PNU PER ML.)	WEIGHT BY VOLUME DILUTION	TOTAL NITROGEN (UNITS PER ML.)
50% Glycerinated	5	10	1-45,000	15.6
	10	10	1-48,400	14.4
	20	10	1-52,500	14.7
	30	10	1-47,400	16.1
	40	10	1-54,000	14.3
	50	10	1-52,800	12.2
Plain	5	10	1-54,500	17.0
	10	10	1-51,600	16.0
	20	10	1-57,000	17.7
	40	10	1-49,800	16.7

Sedimentation.—There was progressive sedimentation during aging, with increasing per cent extraction, in all plain extracts of timothy and ragweed pollens (Table XII). The protective effect of glycerin in the concentration of 50 per cent against the formation of this amorphous sediment was marked.

TABLE XII. SEDIMENTATION IN UNDILUTED POLLEN EXTRACTS

	RAGWEED									TIMOTHY								
	PLAIN				50% GLYCERINATED					PLAIN				50% GLYCERINATED				
Per Cent Extraction	*5*	*10*	*20*	*40*	*5*	*10*	*20*	*30*	*40*	*5*	*10*	*20*	*40*	*5*	*10*	*20*	*30*	*40*	*50*
Original	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 Weeks	+	++	+++	++	0	0	0	0	0	0	+	++	+	0	0	0	0	0	0
4 Weeks	++	+++	++++	++++	0	0	0	0	0	+	++	++	++	0	0	0	0	0	0
6 Weeks	+++	+++	++++	++++	0	0	0	0	0	+	++	++	++	0	0	0	0	0	0
1 Year	+++	+++	++++	++++	0	0	0	0	0	+	++	++	+++	0	0	0	0	0	0

Other observations in this laboratory indicate that glycerin in a concentration of 30 per cent or more is needed to prevent formation of such sediment in undiluted 20 per cent weight by volume ragweed pollen extracts.[22] Sedimentation did not occur in the undiluted 50 per cent glycerinated extracts. However, dilution with unglycerinated buffered saline of fresh or one-year-old 20 per cent weight by volume, glycerinated extracts from 100,000 to 20,000 and 10,000 PNU per milliliter, in some instances, resulted in the formation of a small amount of sediment within a few weeks. No such sedimentation occurred in dilutions of 1,000 PNU per milliliter or weaker concentrations.

A. Ragweed pollen extracts: All glycerinated ragweed extracts remained clear during the one-year period of observation. An amorphous, yellow sediment did appear after two weeks' storage in the plain ragweed extracts and increased in quantity to an apparent maximum at six weeks (Table XII). The original change in appearance was a homogeneous opalescence and turbidity which later separated into layers of yellow, amorphous sediment and clear supernate. The amount of sediment increased as the per cent extraction increased. There were copious quantities in the 20 and 40 per cent weight by volume extracts.

A few studies were made with the amorphous sediment of the 40 per cent weight by volume plain extract after six weeks' storage. The loss in total and PTA nitrogen (0.15 mg. per milliliter), after removal of the sediment from this extract by centrifugation, was accounted for entirely by the nitrogen of the sediment.

Reagin neutralization studies were performed with portions of such sediment after it had been washed five times with cold-buffered saline and redissolved. The whole extract, supernate, and sediment solution were diluted to equal PTA nitrogen concentration. The sediment neutralized against itself, but not against the whole extract. Whole extract neutralized against the sediment in the same range as whole extract against itself.

The whole extract and the supernate had the same end point of neutralization. Thus, there was no apparent loss in activity, even though the isolated sediment had marked neutralizing activity when diluted on an equal PTA nitrogen basis.

Stull and associates[42, 44] found sediment in 9 per cent weight by volume extracts and reported it as probably nonnitrogenous. The present analysis was on a 40 per cent weight by volume extract which may have resulted in 4.5 times more sediment than Stull's extract. The quantities thus involved probably account for their detectability by difference in nitrogen content before and after removal of the sediment from the extract by centrifugation.

Moore and co-workers[30] noted slow, spontaneous, noncrystalline sedimentation in aqueous ragweed extracts and reported the precipitate to be soluble in hot water and to be skin reactive. It was suggested that the reactivity may have been due to contamination with active components from the whole extract.

Chromatographic studies of an unhydrolyzed portion of sediment from two-week-old, plain, 40 per cent weight by volume ragweed extract yielded

no ninhydrin-reacting areas. After hydrolysis, however, there was an amino acid pattern such as that found by Dankner and co-workers[14] and Frankel and associates[18] in their chromatographic analysis of dialyzed residue of plain extract. The change after hydrolysis indicated that this particular sediment contained substances which were protein or polypeptide in nature. The presence of bound carbohydrate was also established for this sediment and this was identified as arabinose by chromatography.

In the present study, no attempt was made to establish activity or to characterize the sediment in the extracts other than that of 40 per cent weight by volume plain ragweed extract, or to identify the possible flavonoid components of the sediments as studied by Heyl,[21] Stevens,[39] Moore and co-workers[30, 31] and Stull and associates.[42, 44]

B. Timothy pollen extracts: The changes in appearance of timothy extracts during storage at refrigerator temperature were somewhat different from those noted in ragweed extracts. An amorphous sediment appeared in the plain extracts after standing two weeks, but only in small amounts, though increasing with increasing concentration of extraction. Sedimentation of amorphous particles did not appear in the glycerinated timothy extracts. However, moderate quantities of a fine, feathery, light yellow, crystalline precipitate appeared in some of the extracts. Since aliquots of the same extract distributed in separate bottles yielded very different amounts of these crystals, it is possible that the surface of the container itself may have influenced the deposition of crystals. These crystals usually appeared after two days' storage at 4° C. and dissolved readily when the extract was warmed to room temperature.

It is believed that these crystals are identical to those described by Moore and Moore[31] as a glucoside, "dactylin," appearing in extracts of orchard grass and timothy pollen. Stull and associates[43] reported such crystals and confirmed Moore and Moore's observations.[31] Studies on the identification and allergenic activity of these crystals are in progress.[23]

COMMENTS

Highly concentrated, potent pollen extracts were obtained by simple, direct extraction methods, without resorting to subsequent concentration procedures. It might be possible to obtain even higher than 50 Gm. per 100 ml. ratio, since, in this study, no plateau or any other evidence of saturation was noted.

The filtrates of the high weight by volume suspensions showed losses up to 56 per cent of the volume of fluid added (Table III). These losses apparently were due to the greater absorption of fluid by the larger amount of dry pollen per unit of fluid added originally for extraction. No attempt was made to recover the absorbed fluid by re-extraction. It was not the purpose of this study to extract the maximum from a particular weight of pollen, but to demonstrate that when increasing quantities of pollen were added to the same volume of fluid, proportionately stronger extracts could be obtained. Furthermore, re-extraction may yield extracts of different qualitative composition. Therefore, for the sake of uniformity, the only variable in these experiments was the weight of pollen per unit of extracting fluid.

Fifty per cent glycerin protected against sedimentation. Plain extracts of high weight by volume ratios became markedly turbid, while glycerinated extracts of the same respective concentrations and pH remained clear. It has been shown previously that, when glycerin was added to freshly prepared pollen extracts to a final concentration of 50 per cent, no differences in reagin neutralization activity of the plain extracts and such glycerinated extracts could be detected.[20]

In this study, the solubility of pollen antigen in 50 per cent glycerinated buffered saline was about 90 per cent of its solubility in buffered saline alone. Equal reagin neutralization activity was observed, when both plain and glycerinated fresh extracts were diluted to the same PTA nitrogen content. This would seem to indicate that the same antigens were extracted by both plain and glycerinated buffered saline, at least within the limitations of reagin neutralization methods used in this study. Multiple antigens and antibodies, and their possible variabilities in reactivity of reaginic sera must be considered in evaluating the results of any experimental studies such as herein presented. The fact remains, however, that potent, highly concentrated pollen extracts can be prepared by direct extraction with either plain or glycerinated fluids, the latter being more practical.

It was not the purpose of this study to infer that there is necessarily complete correlation between allergenic activity and concentration of total and PTA nitrogen and reducing substances, since, in another study,[22] ragweed pollen extract, heated to 56° C. for forty-five minutes, showed loss of reagin neutralizing activity, without any loss of PTA nitrogen or bound carbohydrate. Using freshly prepared, unmodified extracts, however, the quantitative relationship between concentration of these components and reagin neutralization activity in relation to weight by volume extraction ratio was distinctly proportional.

There were certain observations in these experiments that remain unexplained.

Four-week-old ragweed extracts, both plain and glycerinated, showed an increasing neutralizing capacity as the concentration of extraction increased (Table VII). This was less evident at nineteen weeks (Table VI) and even less demonstrable after one year (Table VIII).

Timothy pollen extracts, both plain and glycerinated, showed no increased neutralizing capacity at four weeks, as was the case with ragweed. One-year-old plain timothy extract actually showed a decreasing neutralizing capacity as the concentration increased, although there was a distinct proportional linearity of all glycerinated extracts after one year.

The possibility that unequal dispersion of sediment particles during dilution of the timothy extract might account for this observation is doubtful, since the plain ragweed extracts containing even greater amounts of sediment did not show this phenomena. Further study in the comparison of the chemical and biologic properties of the timothy and ragweed sediments might clarify these observations.

The limitations of end point evaluation in reagin neutralization, however, may not permit definitive end point determinations in a narrow range of 25 protein nitrogen units or less.

The fact remains, however, that more active ragweed and timothy pollen extracts were prepared by increasing the weight by volume concentration of extraction.

SUMMARY

1. Highly concentrated timothy and low ragweed pollen extracts have been prepared by direct extraction.

2. Extracts of timothy and low ragweed pollens, prepared in increasing weight by volume ratios, from 5 to 50 per cent, showed a distinct proportionality, indicating extraction in linear ratios, as measured by total and phosphotungstic acid-precipitable nitrogen content, concentration of reducing substances, and reagin neutralization activity.

3. The use of glycerinated extracting fluids was necessary in the preparation of such highly concentrated and potent extracts to avoid sedimentation and to preserve activity.

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FRACTIONATION OF POLLEN EXTRACTS

II. Chemical and Paper Chromatographic Investigation of Ragweed Pollen Extracts

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A PREVIOUS report from this laboratory recorded observations on the separation of the dialyzable components of ragweed extract dialysate by the method of partition paper chromatography.[1] The data incorporated in that report were concerned, in part, with determinations of the biologic activity and the amino acid content of some of the fractions obtained by one dimensional paper chromatography with water saturated lutidine (2, 4-dimethylpyridine). Later studies with the resulting components of dialysate revealed that lutidine alone was capable of provoking positive skin reactions in ragweed-sensitive subjects. The fractionation of ragweed extract dialysate was, therefore, repeated, using a nonskin reacting solvent, methyl cellosolve (ethylene glycol monomethyl ether). The present report is concerned with this separation and with the analysis of the resulting fractions. When it was found that further manipulation of the fractions resulted in successive breakdown and loss of material, a preliminary study was made of the nondialyzable residue, certain data of which are included in this report.

METHODS AND RESULTS

Low ragweed pollen was extracted and separated into its dialyzable and nondialyzable components by methods described in the earlier publication.[1] The present procedure differed only in that water was used throughout instead of buffer for the initial extraction of the pollen, and the whole extract was concentrated twentyfold by vacuum distillation at a temperature which never exceeded 35° C.

A. *Dialysate Fractions.*—

I. Lutidine: Paper chromatographic fractions of the dialyzable portion of ragweed extract were prepared in large quantities by the Nepera Chemical Co., Inc. of Yonkers, New York.

Dialysate of ragweed extract was stripped across a short edge of a large sheet of filter paper and developed one dimensionally using lutidine. Spraying

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a guide strip of the dried paper with ninhydrin revealed four polypeptide bands (called A, B, C, D) while spraying another guide strip with aniline hydrogen oxalate revealed the presence of one carbohydrate containing band (called CHO). After lyophilization, the eluted fractions A, B, C, and D were obtained as brown powders and fraction CHO as a dark brown syrup. All fractions were skin reactive upon intradermal test in ragweed-sensitive subjects, and their total nitrogen and amino acid contents were similar to those of the fractions studied previously.

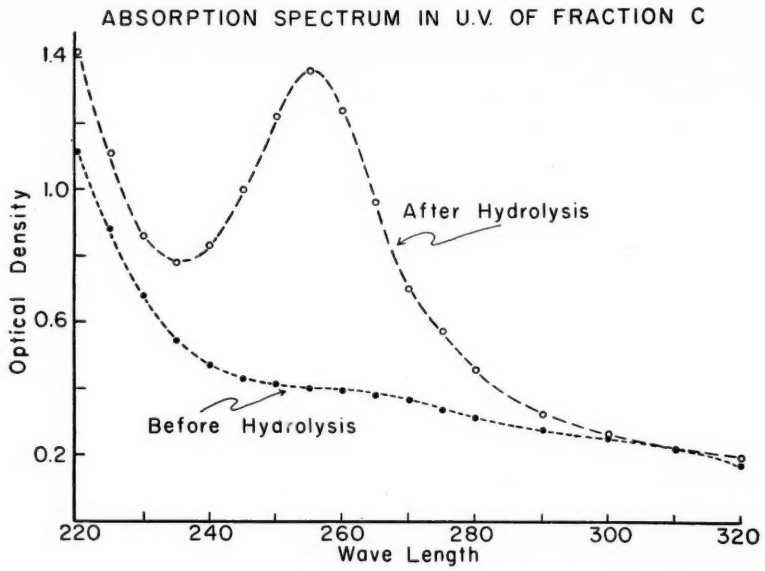

Fig. 1.

A portion of each fraction was placed in sealed tubes and hydrolyzed with 6 N hydrochloric acid at 100° C. for eighteen hours. Despite such drastic treatment, fractions A, C, and CHO retained skin reactivity, while fractions B and D became inactive. Ultraviolet absorption spectra (Model DU Beckman) obtained before and after hydrolysis revealed the appearance of an absorption band at 260 mμ in only those hydrolyzed fractions which retained skin reactivity. Fig. 1 shows curves of fraction C before and after hydrolysis, and is typical of the behavior of all three fractions.

These unexpected results prompted an investigation of lutidine alone, and it was found that the absorption spectrum of a very dilute solution of this material was very similar to those obtained with the hydrolyzed fractions. Furthermore, positive skin reactions were obtained when lutidine alone was tested intradermally in ragweed-sensitive subjects. This may well be correlated with the findings of Winkenwerder, Buell, and Howard[2] who showed that nitrogenous materials derived from nucleic acid exhibited skin reactivity in ragweed-sensitive subjects. It seemed possible that lutidine had combined chemically with the fractions during separation and was itself responsible for

the biologic activity previously attributed to the fractions. For this reason the fractionation of dialyzable ragweed extract was repeated with the aid of a nonskin reacting solvent. Methyl cellosolve, which had been reported as giving Rf values for amino acids similar to those with lutidine,[3] was chosen for this fractionation after preliminary study revealed it was not itself skin reactive.

II. Methyl cellosolve: The cellosolve separation was accomplished by processing 150 sheets of water-washed filter paper each of which had been streaked with 110 to 125 mg. of concentrated dialysate. These sheets were then developed, one dimensionally with 90 per cent aqueous methyl cellosolve in the same manner previously employed with lutidine. Although lutidine had separated four ninhydrin reacting bands and one carbohydrate containing band, methyl cellosolve separated eight ninhydrin reacting bands and one carbohydrate containing band.

TABLE I. NITROGEN AND CARBOHYDRATE ANALYSES OF CELLOSOLVE FRACTIONS

SAMPLE	Rf* (CELLOSOLVE)	% TOTAL NITROGEN	% BOUND CARBOHYDRATE†
0	0.00	6.1	0.0
1	0.10	--	--
2	0.30	6.4	0.0
2A	0.42	6.3	2.3
3	0.49	3.4	0.0
3A	0.58	4.1	7.0
4	0.70	6.3	0.0
5	0.87	7.5	9.2
CHO	0.93	1.6	21.0 (free)

*Rf represents the fraction of the distance traveled based on the distance of the solvent which is taken as 1.

†The bound carbohydrate present in the fractions was arabinose, while the free carbohydrate in fraction CHO was a mixture of glucose and fructose.

Table I shows the results of nitrogen and carbohydrate analyses performed on the cellosolve fractions. The nitrogen determinations were done by semimicro Kjeldahl, the carbohydrates by the method of Nelson.[4] The separation between some of the bands (2A and 3 and 5 and CHO) was not great as indicated by their Rf values. Nevertheless, elution of the fraction was carried out only when a discrete white area was present between the extremes of the blue bands made by ninhydrin reaction with the separate fractions. No blue color appeared in the area called CHO, but it yielded a typical brown band for carbohydrate after treatment with aniline hydrogen phthalate. The nitrogen values of the ninhydrin reactive bands ranged from 3.4 to 7.5 per cent while the carbohydrate content of these fractions ranged from 0 to 9.2 per cent and, where present, was exclusively bound arabinose. The 21 per cent carbohydrate content of the CHO fraction was entirely in the free form and was identified by paper chromatography as glucose and fructose.

Each of the eight ninhydrin reacting bands after removal from the paper proved to be skin reactive. Rechromatography of these bands, however, with 80 per cent aqueous isopropanol, for further purification, resulted in separation

of each band into several smaller bands. This successive breakdown into smaller units is indicative of considerable instability of the isolated fractions. This was further confirmed by the fact that several amino acids appeared on two dimensional chromatograms of all the fractions even before they were subjected to acid hydrolysis. The major portion of each fraction obtained by isopropanol rechromatography was nevertheless analyzed for amino acid composition before and after acid hydrolysis.

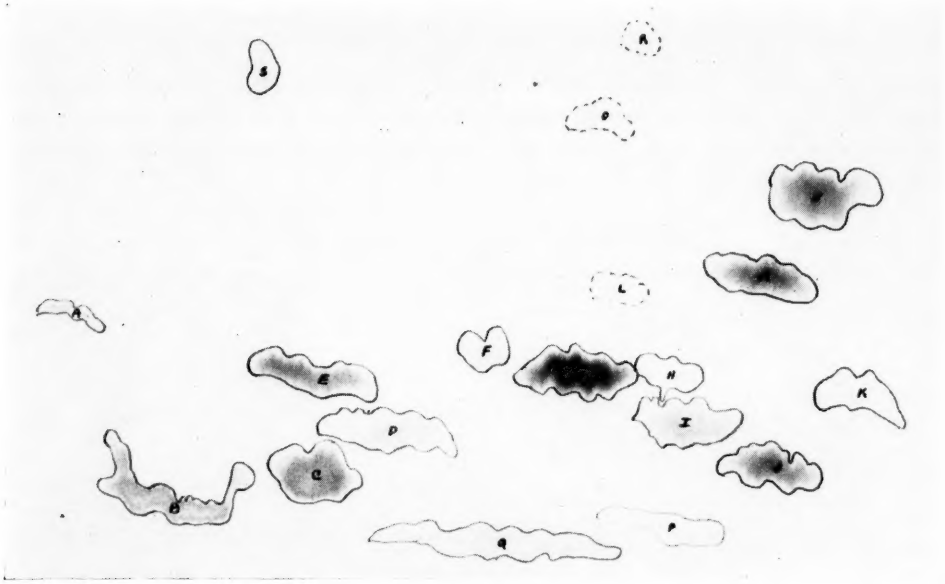

Fig. 2.—Dialysate of ragweed hydrolyzed 3 mg.

Key to Symbols: *A*, Cystine; *B*, Aspartic Acid; *C*, Glutamic Acid; *D*, Glycine; *E*, Serine; *F*, Threonine; *G*, Alanine; *H*, Hydroxyproline; *I*, Histidine; *J*, Gamma-aminobutyric Acid; *K*, Proline; *L*, Methionine; *M*, Valine; *N*, Leucine; *O*, Tyrosine; *P*, Arginine; *Q*, Lysine; *R*, Over Tyrosine; *S*, Unknown; *W*, Beta-alanine.

Two dimensional chromatograms of these partially purified fractions revealed similar but not identical patterns for all the fractions each of which contained fewer amino acids than had the unfractionated dialysate (Fig. 2). Common to all fractions was the presence of aspartic and glutamic acids, glycine, serine, alanine, and a trace of cystine. The rechromatographed cellosolve fraction 3, after acid hydrolysis, contained a rather large quantity of gamma-aminobutyric acid and a trace of hydroxyproline (Fig. 3). Cellosolve fraction 4, however, showed an undetectable amount of these amino acids but a large amount of valine and a trace of leucine (Fig. 4). Histidine, which was very evident in the unfractionated dialysate, did not appear in either of these fractions. In several of the unhydrolyzed fractions glutamine was detected and in two of them asparagine was present.

The free carbohydrates present in the unfractionated dialysate were shown by paper chromatography to be glucose and fructose. This was further confirmed by the preparation of the glucosazone and the use of spraying

reagents specific for ketoses. Brown and Benotti[5] prepared a glucosazone from Norite A decolorized ragweed pollen extract, which is not capable, however, of distinguishing fructose from glucose. The fact that glucose and fructose migrate at the same rate in the developing solvents used in the present study, accounts for the fact that both sugars appeared in the single band labeled CHO.

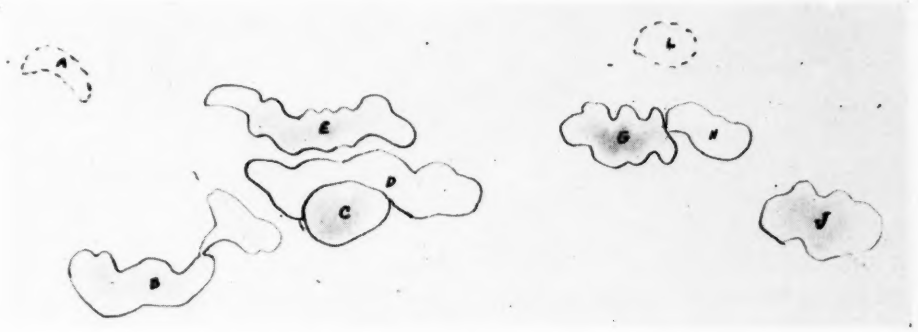

Fig. 3.—Cellosolve fraction 3 rechromatographed 80 per cent isopropanol hydrolyzed 3.6 mg. (For Key to Symbols, see opposite page.)

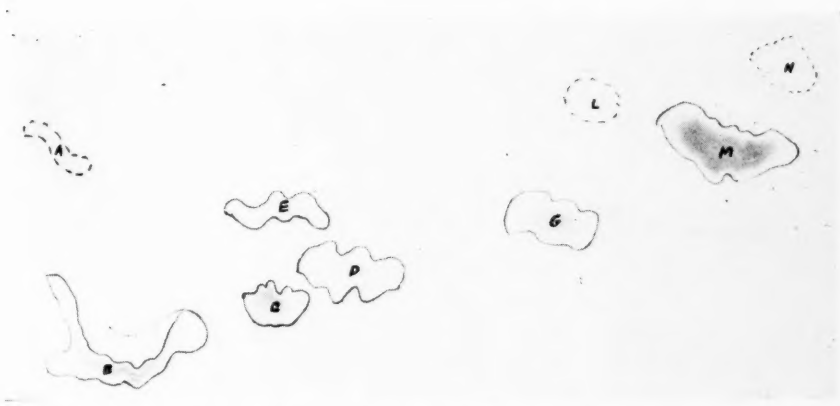

Fig. 4.—Cellosolve fraction 4 rechromatographed 80 per cent isopropanol hydrolyzed 3 mg. (For Key to Symbols, see opposite page.)

III. Alcohol and acetone: Several investigators[10, 16-19] have succeeded in recovering active fractions from whole ragweed by precipitation with alcohol and/or acetone. Advantage was taken of this observation in an attempt to fractionate whole dialysate in order to obtain a less complex mixture for subsequent paper chromatographic separation. It was found that nearly all of the skin reactivity present could be precipitated at 60 per cent concentration of either solvent. Upon re-solution of the precipitate in water there remained a white, insoluble residue which proved to be calcium phosphate. Moreover Brown's observation that all phosphorus present in ragweed extract was inor-

ganic[5] was confirmed. Nitrogen, carbohydrate, and amino acid analyses, as well as skin reactivity determinations were done on several fractions obtained by serial precipitations with increasing concentrations of either alcohol or acetone. The principal amino acids appearing on two dimensional paper chromatograms of these isolated precipitates were essentially the same as those listed for the cellosolve fractions with the exceptions that large amounts of histidine and only very faint traces of hydroxyproline and proline were present.

Table II shows the analysis of a typical set of fractions obtained by acetone precipitation of dialysate. The chemical similarity to the original dialysate of most of the active fractions suggests the possibility that the precipitated fractions are the same except for polymer size. Two of the fractions produced larger wheals on direct intradermal testing than did the original dialysate. The presence of unusual amounts of histidine may result from its relative insolubility in fairly high concentrations of organic solvents. Of considerable interest is the fact that one of the more active fractions (40 per cent acetone insoluble) showed no detectable carbohydrate. From these data, it would appear that the allergenic activity of the various fractions may not be proportional to the carbohydrate content as was claimed for the hydrochloride fractions obtained by Rockwell.[9]

TABLE II. ACETONE FRACTIONS OBTAINED FROM DIALYSATE

SAMPLE	HISTIDINE*	% TOTAL NITROGEN	% BOUND CARBOHYDRATE	ACTIVITY†
Dialysate	L	6.5	7.6	+++
40% Acetone insoluble	L	5.5	0.0	++++
60% Acetone insoluble	L	6.3	10.4	++++
70% Acetone insoluble	L	6.6	9.4	++
100% Acetone soluble	0	2.7	8.6	0

*L under histidine signifies a large amount of histidine as seen on two dimensional paper chromatograms.

†Intradermal tests with solutions of 5 mg. per milliliter in buffered saline. The number of plus signs represents an average of many determinations and they are relative to unfractionated dialysate at +++.

Within the limits of skin testing procedures, the specific activity of each of the acetone fractions could not be increased by repeated precipitation. For this reason and because there was progressive loss of material with each succeeding purifying procedure this approach to isolation of an active fraction was discarded, and the next phase of study was concerned with an analysis of nondialyzable fractions.

B. *Residue Fractions (Nondialyzable).*—The nondialyzable portion of the ragweed extract retained by far most of the skin reactivity. Neither ninhydrin nor aniline hydrogen phthalate reacting components appeared on chromatograms of unhydrolyzed residue. Both types of constituents, however, appeared in large amounts after acid hydrolysis. Paper chromatographic analysis of 1 N acid hydrolysates revealed the presence of very large amounts of arabinose in agreement with the findings of Rockwell[7] and Stull, and co-workers.[8] Fig. 5 shows that the amino acids appearing after 6 N acid hydrolysis were cystine, aspartic

and glutamic acids, glycine, serine, threonine, alanine, valine, leucine, arginine, lysine, beta-alanine, gamma-aminobutyric acid, methionine, traces of tyrosine and an unknown material appearing above tyrosine in the approximate position of one of the iodotyrosines. Extremely large amounts of hydroxyproline and proline were present in all preparations and in all subsequent fractions obtained from them although the yellow color characteristic of these compounds is not clearly evident in the black and white reproduction.

The exhaustively dialyzed residue contained, by weight, 4.5 per cent total nitrogen which was entirely precipitable by phosphotungstic acid, and 40.8 per cent bound carbohydrate determined by the method of Nelson and by the orcinol method for pentoses. Qualitative analysis of the sugar component by paper chromatography showed the presence of arabinose alone.

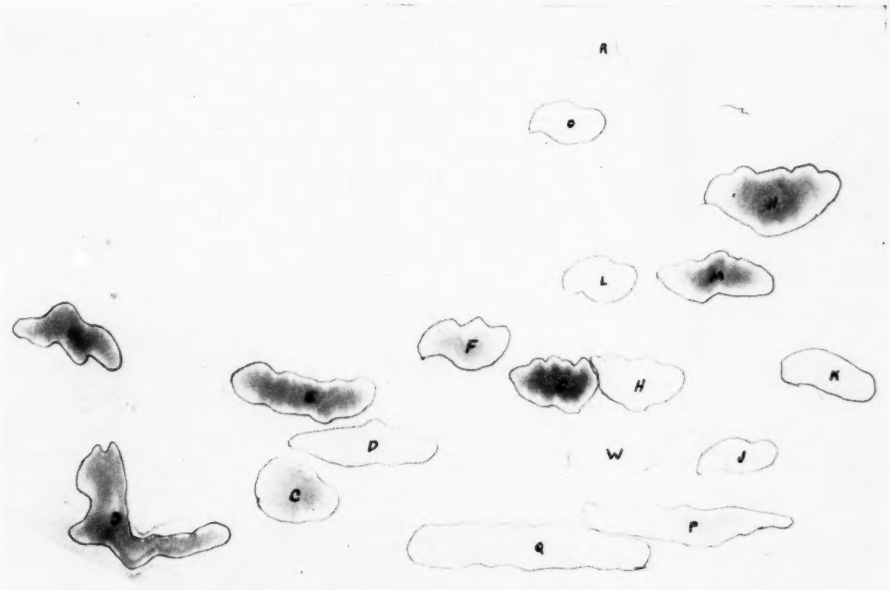

Fig. 5.—Nondialyzable residue of ragweed hydrolyzed 3 mg. (For Key to Symbols, see page 36.)

Several fractionation procedures were used in order to determine whether the biologic activity was due to a polypeptide, a carbohydrate, or possibly to an intact polypeptide-carbohydrate complex. The limit of chemical manipulation tolerable to the active components was evaluated in the process of attempting to isolate the purest possible active material. Several investigators have attempted this sort of fractionating procedure but without much success. The carbohydrate fractions isolated by Black[10] and Caulfeild[11] each contained appreciable amounts of nitrogen. Rockwell[9] isolated five fractions each consisting of a flavonol-polypeptide-carbohydrate complex and each exhibited biologic activity, but it was not proved that the activity was dependent on the entire complex or any part of it. Stull[12] obtained by ammonium sulfate precipitation a carbohydrate-free fraction which retained activity, but this was not confirmed in the present

study. Rockwell[13] reports that the pollen antigen was destroyed by alkalies, yet in this laboratory it was possible to prepare a fraction by alkaline hydrolysis at 37° C. which contained 74 per cent bound carbohydrate and only 1.5 per cent nitrogen, and which retained about half of the activity exhibited by the original residue (Table III). It was found that activity could be preserved even after treatment with 1 N acid or 1 N alkali if the temperature was maintained below about 40° C. A few fractions were obtained free of carbohydrate, but the treatment required hydrolysis with 1 N hydrochloric acid in boiling water for one hour which destroyed the skin reactivity. In no case was it possible to obtain from residue an active fraction which was completely free of either nitrogen or carbohydrate.

TABLE III. ANALYSES OF FRACTIONS FROM THE NONDIALYZABLE PORTION OF RAGWEED POLLEN EXTRACT

SAMPLE	% TOTAL NITROGEN	% BOUND CARBOHYDRATE	ACTIVITY*
Untreated residue	4.5	40.8	++++
Insoluble 50% saturated ammonium sulfate	7.7	22.7	+++
Insoluble 100% saturated ammonium sulfate	5.9	44.9	+++
Insoluble 69% aqueous acetone	7.7	20.8	++++
After HCl hydrolysis (hot)	10.4	00.0	0
After NaOH hydrolysis (warm)	1.5	74.4	++
Lead precipitation (Caulfeild)	3.4	55.9	++
Phenol extraction (Morgan)	2.1	72.4	++

*Each solution was adjusted to 100 PNU per milliliter on the basis of the total nitrogen which, in these experiments, was the same as the PTA nitrogen.

I. Ammonium sulfate: Two fractions were obtained which were insoluble, respectively, in 50 per cent and 100 per cent saturated ammonium sulfate. Each fraction was reprecipitated with the concentration of ammonium sulfate used for initial precipitation until the precipitate was of constant carbohydrate and nitrogen composition. This was accomplished after three reprecipitations, but each fraction so obtained was dark brown in color and contained carbohydrate By contrast, Stull's Fraction I obtained after ten reprecipitations, was nearly colorless and contained no carbohydrate.[12]

The 50 per cent and 100 per cent ammonium sulfate insoluble fractions obtained in this study were of approximately equal skin reactivity on direct intradermal testing. The 50 per cent insoluble fraction, however, contained more nitrogen and less carbohydrate than did the 100 per cent fraction. Each fraction was probably a complex mixture as evidenced by its electrophoretic inhomogeneity in phosphate buffer at pH 6.7. The fraction insoluble at 50 per cent salt saturation contained 7.7 per cent total nitrogen and 22.7 per cent bound arabinose, while the fraction insoluble at 100 per cent salt saturation contained 5.9 per cent total nitrogen and 44.9 per cent bound arabinose.

II. Acetone precipitation: A light tan powder was obtained by bringing the aqueous solution of residue to 90 per cent acetone concentration. This powder exhibited the same activity as the original solution. Further fractionation of the powder with 60 per cent, 70 per cent, 80 per cent, and 89 per cent acetone produced materials of similar skin reactivity and chemical composition

with the exception of the 80 per cent soluble, 89 per cent insoluble fraction which had a bound carbohydrate higher than the others. The nitrogen values ranged from 7.2 per cent to 7.9 per cent and the bound arabinose from 19.3 per cent to 21.7 per cent, the 89 per cent fraction having 31.3 per cent bound carbohydrate.

III. Acid hydrolysis: It had been repeatedly demonstrated in the course of this study that the bound carbohydrate of ragweed pollen is completely hydrolyzed by 1 N hydrochloric acid in boiling water for one hour. Under these conditions, very little hydrolysis of protein occurs. Accordingly this procedure was attempted in order to obtain a carbohydrate-free fraction. It was indeed possible to obtain such a fraction, but this was accompanied by an expected destruction of skin reactivity. The faintly colored material which precipitated during the hydrolysis contained 10.4 per cent nitrogen and no carbohydrate. Milder methods of hydrolysis, employing weaker acid and lower temperatures failed to produce a fraction which was entirely free of carbohydrate although it was possible to retain about one-half of the original skin reactivity.

IV. Alkaline hydrolysis: It seemed possible that the bound arabinose in ragweed extract was present as a polysaccharide. If present as such a polymer, the carbohydrate should resist hydrolysis with alkali while any polypeptides present could be expected to be degraded under these conditions. Therefore, the aqueous residue was made 1 N with sodium hydroxide and allowed to stand in a warm room at 37° C. for twenty-four hours. The hydrolysate was neutralized and reduced in volume in vacuo. Acetone was added to 90 per cent, the precipitate removed, washed with 90 per cent acetone, 100 per cent acetone, and dried under vacuum. There was present 1.5 per cent nitrogen, 74.4 per cent bound arabinose and approximately half of the original skin reactivity representing the best separation with retention of activity in the entire series. Since the carbohydrate component was in the bound state even though there was such a small amount of nitrogen present, it is evident that the arabinose is present as a polysaccharide rather than in the monomolecular form as pictured by Rockwell.[7] The small amount of nitrogen was shown by paper chomatography to be in the form of amino acids rather than nitrogen-containing carbohydrates.

V. Miscellaneous: Other methods were employed in order to obtain a carbohydrate fraction, but none produced a totally nitrogen-free fraction. The method of Caulfeild[11] produced an active fraction containing 3.4 per cent nitrogen and only 55.9 per cent bound arabinose. Some of the methods used by Morgan[14] in his fractionation of a polypeptide-polysaccharide antigen were attempted. One procedure called for extraction with phenol which, when applied to residue, produced an active fraction containing 2.1 per cent nitrogen and 72.4 per cent arabinose. Again it was shown that the nitrogen was present as amino acids rather than amino sugars, and that the only carbohydrate detectable was arabinose.

CONCLUSION

All of the fractions obtained from residue by the various methods used agree in general with the results obtained by Rockwell,[9] namely, that these are

polypeptide-carbohydrate-pigment complexes, with the exception that the carbohydrate component appears to be an araban rather than arabinose. Only in the dialyzable portion, however, was it possible to obtain an active fraction which was completely free of carbohydrate. Regardless of the fractionation procedures employed, both paper chromatographic and chemical, there was never obtained from residue an active fraction which was entirely free of either nitrogen or carbohydrate. This does not necessarily imply that the combination of polypeptide and polysaccharide is essential for allergenic activity, but rather that suitable methods for a complete separation result in a destruction of biologic activity.

Augustin[15] has stated that the active components of the grass pollens are nondialyzable. If the active materials in dialysate are truly degradation products from residue and since CHO free fractions were obtained from dialysate, then the separation of the components of the active fractions of residue should be possible.

It would seem that paper chromatography and the chemical procedures used in this study are unable to define the chemical nature of ragweed activity without the addition of such other methods as resin column extractions and electrophoresis by any of the varied techniques available.

SUMMARY

1. The solvent for paper chromatographic separations of skin reactive allergens must be chosen with great care. Lutidine can combine chemically with certain compounds and is in itself capable of producing skin reactions.

2. Fractions very similar to those previously obtained with lutidine were separated by use of a nonskin reactive solvent, methyl cellosolve. Such fractions exhibited skin reactivity. From this it has been concluded that the originally separated lutidine fractions were actually active components of dialysate.

3. Activity in the dialysate of ragweed extracts appears to be due to breakdown of originally larger particles, as shown by the extreme lability of the fractions isolated by paper chromatography.

4. Nearly all of the skin reactivity present in dialysate was precipitable by making an aqueous solution 60 per cent with respect to acetone.

5. Chemical fractionation of the nondialyzable portion of ragweed extract failed to produce an active fraction which was completely free of either protein or carbohydrate, respectively. Warm alkaline hydrolysis and phenol extraction came closest to producing such a fraction although the specific activity was reduced in both cases. Dialysate did, however, produce a few skin reactive carbohydrate-free fractions.

6. The active components appear to consist of a protein-carbohydrate-pigment complex, the carbohydrate component being a polysaccharide of arabinose.

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ACTH AND CORTISONE IN THE TREATMENT OF ASTHMA

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TWO years ago in our initial report[1] of the use of ACTH and cortisone in asthma, a series of eleven cases was used as the basis of our study and the longest period of treatment in any one case was eleven months. The present study comprises 60 cases seen at the New York Hospital and in private practice, and the longest period of treatment in any single case has been thirty-nine months.

The usefulness of the hormones in the treatment of asthma, deleterious complications that may result from their continued use, and proper dosage have been studied.[1-6] These questions, as well as alternation of therapy between HP Acthar gel and cortisone, the relation of therapy to adrenal function as shown by the Thorne test, the relation of therapy to respiratory function, and the relation of hormone therapy to other forms of management were investigated in this study.

Selection of cases for hormone therapy has been based primarily on the failure of patients to respond favorably to the usual management, and on the diagnosis of severe status asthmaticus. Hormone therapy was continued where favorable progress seemed contingent upon the maintenance of such therapy. In many of the patients gradual withdrawal of the hormone resulted in no flare-up of the asthmatic state. In the selection of our cases we excluded patients with psychoses, tuberculosis, diabetes, and ulceration of the gastrointestinal tract.

Data pertaining to sex, age, duration, and type of disease, as well as complications observed in these patients, are summarized in Table I. It will be seen that twenty-five patients were male and thirty-five were female; the ages varied from 6 to 70 years; thirty-five (59 per cent) were in the "older" age group of 40 to 70 years; the duration of disease prior to the onset of therapy varied from six months to fifty years; thirty-eight (63 per cent) had had asthma for at least one-third of their entire life span; definite sensitivities proved by skin test and related to asthma were present in forty-six cases (77 per cent);

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Cortisone was supplied without charge by Merck and Company.

HP Acthar gel was supplied without charge by The Armour Laboratories, Chicago, Illinois.

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TABLE I. GENERAL CONDITIONS OF PATIENTS STUDIED

	TOTAL	SEX		AGE		LIFE SPAN OF ASTHMA		SKIN TEST		INFECTION		PSYCHOSOMATIC	
		MALE	FEMALE	OVER 40 YEARS	UNDER 40 YEARS*	OVER ⅓ OF LIFE	UNDER ⅓ OF LIFE	+	−	+	−	INCREASED	NOT INCREASED
Number	60	25	35	25	35	38	22	46	14	41	19	34	26
Per cent	100	41	59	41	59	63	37	77	23	68	32	56	44

*Six patients were below 14 years of age.

chronic infectious sinusitis of the hyperplastic type, often with nasal polyps, was present in forty-one (68 per cent); psychoneurotic manifestations to an abnormal degree were present in thirty-four (56 per cent). Of this series of patients, seven have died, but only two of them were receiving the hormones at the time of death.

In this series of sixty patients, thirty continued to require some maintenance with one or the other of the hormones, following the institution of this form of treatment. The remaining thirty have required hormone therapy only for intermittent relapse. The largest amounts of hormone naturally have been given to the patients requiring maintenance therapy. Cortisone was administered orally except in a few instances where rapid administration was indicated. ACTH was given, for the most part, intramuscularly and the preparation used was HP Acthar gel.

In Table II, the total doses of cortisone exceeding 10 grams, given to fourteen patients, and the duration of therapy are reported. The largest amount of cortisone administered to an individual patient was 62 grams, given over a period of thirty-six months, and the longest period of observation in this group was thirty-nine months.

Table III summarizes the results noted in eleven patients treated with HP Acthar gel in amounts exceeding 2,000 units, injected over periods of time varying between seven and thirty-four months. As presented in Table III,

TABLE II. DOSAGE AND DURATION OF CORTISONE THERAPY (10 OR MORE GRAMS)

PATIENT NUMBER	TOTAL DOSE (GRAMS)	DURATION (MONTHS)
1	62.0	36
2	48.0	28
3	37.8	33
4	33.8	39
5	29.6	16
6	28.0	16
7	22.4	15
8	19.2	29
9	17.6	9
10	13.6	13
11	13.0	11
12	12.5	14
13	11.3	6
14	10.6	11

TABLE III. DOSAGE AND DURATION OF ACTHAR GEL THERAPY (OVER 2,000 UNITS)

PATIENT NUMBER	TOTAL DOSE (UNITS)	DURATION (MONTHS)
15	7780	24
16	6180	10½
14	6180	15
17	5110	34
18	4000	10
19	3560	12
20	3040	20
4	2640	4
21	2350	6
1	2200	7
8	2200	7

patient 15 received a total of 7,780 units of the hormone in twenty-four months, and patient 17 was treated with Acthar gel for thirty-four months.

In Table IV, data are compiled from 7 patients who were given combined or alternating treatment with cortisone and HP Acthar gel.

TABLE IV. CORTISONE AND ACTHAR GEL THERAPIES (COMBINED OR ALTERNATING)

PATIENT NUMBER	TOTAL DOSE		DURATION (MONTHS)
	CORTISONE (GRAMS)	ACTHAR GEL (UNITS)	
4	33.8	2640	39
15	7.5	7780	24
14	10.6	6180	15
19	6.1	3560	12
1	62.0	2200	36
8	19.2	2200	29
20	9.6	3040	20

COMPLICATIONS

Complications attributable to the use of the hormones were very few. Patient 17 developed fluid retention with cardiac decompensation, quickly relieved by reducing the dosage of the hormone, minimum salt diet, and Mercuhydrin as a diuretic; patient 3 developed benign adenoma of the breast; patient 29, with asthma and Raynaud's disease, developed thrombosis of the peripheral arteries; patient 53 became depressed and developed a coronary thrombosis, unrecognized, from which he recovered while receiving cortisone intramuscularly. Moderate weight increases and the appearance of a "moon face" were noted occasionally but promptly subsided upon reduction of hormone dosage.

Radiographs of the bones of several patients taking the largest amounts of the hormone have failed to reveal any abnormal degree of osteoporosis. Acne and furunculosis have not been prevalent. None of the patients developed peptic ulcer or had hemorrhage from the gastrointestinal tract.

SURGERY DURING HORMONE THERAPY

Sixteen cases underwent some form of surgery during hormone therapy. Four patients had nasal polypectomies; seven had Caldwell-Luc operations;

tonsils and adenoids were removed in two; one had a cervical laminectomy; one had a sub-mucous resection; and one a dilatation and curettage. One patient delivered twins successfully after arriving in the hospital critically ill with status asthmaticus. She received intravenous ACTH before and after delivery.* No complications were observed in any of these cases. Our procedure follows the precautions suggested by Salessa, Bennett, Keating, and Sprague[7] in regard to the preoperative and postoperative management of patients who have had previous treatment with cortisone or ACTH.

MORTALITY

In this series, seven patients died; all were men between the ages of 48 and 70 years. The essential data on these seven patients are presented in Table V. In summary, only two of the seven fatalities occurred during administration of small amounts of hormones. One of them (patient 56) can be explained adequately by the chronic pulmonary disease and pneumonia diagnosed in this case. The other case (patient 14, who had received large doses of ACTH and cortisone over a long period of time) resembled to some extent a condition known as Hamman-Rich syndrome.[8]

TABLE V. GENERAL DATA ON FATALITIES

PATIENT NUMBER	AGE (YEARS)	CAUSE OF DEATH	HORMONE THERAPY AT TIME OF DEATH
56	59	Chronic pulmonary fibrosis, pneumonia, cardiac decompensation	Small doses of cortisone
14	54	Cardiac decompensation and cardiac pulmonary syndrome	Small doses of cortisone and HP Acthar gel
54	53	Cardiac decompensation and cardiac pulmonary syndrome	None
28	70	Carcinoma of rectum	None
53	54	Acute coronary thrombosis	None (for 9 days)
34	48	Status asthmaticus*	None (for 4½ months)
55	49	Periarteritis nodosa	None (for 6 months)

*This patient was not under our observation at the time of death.

TESTS FOR ADRENAL FUNCTION

In a number of cases studies were made of the effect of long continued dosage of cortisone on the change in the eosinophil count following ACTH (Thorne test), or a modification of Thorne test using HP Acthar gel. It would seem that patients may take large amounts of cortisone and that following cessation there may be evidence of adrenal suppression for a few days which will then gradually disappear. Two cases are offered as typical examples.

Patient 7, a 54-year-old woman with asthma for twenty years, received cortisone from Jan. 29, 1952, until March 15, 1953—a total of 20.3 grams. Forty-eight hours later, a Thorne test showed 178 eosinophils per cubic millimeter before, and 177 after, ACTH but on the seventh day following cessation of cortisone treatment the Thorne test showed 276 eosinophils per cubic millimeter before ACTH and 141 after.

*This patient, one year later, while still on hormone therapy, became pregnant, continued to take cortisone, and in March, 1954, had a normal delivery of a normal infant. Another patient became pregnant while on cortisone therapy, continued it throughout her pregnancy, and had a normal delivery of a normal child in April, 1954

Patient 1, a man 57 years of age, with asthma for eight years, was given a total of 12 grams of cortisone from Sept. 25, 1950, until June 8, 1951. On that day a Thorne test showed 220 eosinophils per cubic millimeter before ACTH and 248 after. In this instance, there was no cessation of cortisone before the Thorne test was performed. On Feb. 4, 1953, having had a total of 62.3 grams of cortisone in the previous thirty months, the Thorne test showed 294 eosinophils per cubic millimeter before ACTH and 248 after. Cortisone therapy was discontinued on Feb. 4, 1953, and the patient was given HP Acthar gel, 40 units intramuscularly, three times a week. On Feb. 10, 1953, twenty-four hours after the last dose of 40 units of HP Acthar gel, the eosinophil count was 3 per cubic millimeter, and on April 10, 1953, twenty-four hours after administration of 30 units of HP Acthar gel, the eosinophil count was 6 per cubic millimeter.

Satisfactory clinical response to the hormones was noted, even though the Thorne test failed to indicate adrenal response, as evidenced by a drop in the number of circulating eosinophils following administration of HP Acthar gel.

Patient 15, a 48-year-old man with asthma of thirteen years' duration, received HP Acthar gel from Oct. 12, 1951, until March 2, 1953. During the period of more than sixteen months, a total of 6,500 units was administered, and with 30 units of HP Acthar gel two times a week, his asthma was satisfactorily controlled. His eosinophil count on March 2, 1953, was 31 per cubic millimeter. Starting March 4, 1953, he was given cortisone in gradually increasing doses, while HP Acthar gel was gradually decreased. Between this time and June 17, 1953, he received 6 grams of cortisone and his asthma continued well controlled. On June 19, 1953, forty-eight hours after stopping cortisone, his asthma was worse. Thorne test showed 654 eosinophils per cubic millimeter before ACTH, and 693 after. Four days later (or six following cessation of cortisone) a Thorne test showed 633 eosinophils per cubic millimeter before ACTH, and 638 after. He was then given HP Acthar gel, 40 units every other day, and his asthma improved. On Aug. 12, 1953, he was again put on cortisone and has continued with satisfactory control of his asthma on 37.5 mg. of cortisone a day.

It would seem that caution must be exercised in attributing too much importance to the Thorne test as a sole criterion of adrenal function or as a criterion of clinical response to either of the hormones in the management of asthma.

RESPIRATORY FUNCTION STUDIES

Respiratory function studies were done in the Cardio-Pulmonary Laboratory of the New York Hospital, Dr. Daniel Lukas in charge. Maximum breathing capacity before and after inhalation of Vaponefrin was determined at least one time on twenty-four patients of this series. We do not wish to give an elaborate description of results in this article, but certain general conclusions can be stated. If Vaponefrin causes an appreciable increase in the maximum breathing capacity, HP Acthar gel or cortisone in proper dosage will probably produce the same effect, but whereas Vaponefrin causes an improvement in re-

spiratory function for a matter of minutes or hours, the hormones may maintain this improvement day after day, and in some cases with increasingly better function as time goes on.

Patient 1, a 57-year-old man with asthma for for eight years, before receiving the hormone, had a maximum breathing capacity of 34.2 per cent of the predicted normal before Vaponephrin, and 74.7 per cent after inhalation. On Sept. 15, 1950, eight days after receiving HP Acthar gel, his maximum breathing capacity was 59.1 per cent before, and 74.7 per cent after, inhalation of Vaponefrin. Two weeks after receiving HP Acthar gel his maximum breathing capacity was 112.8 per cent of normal before Vaponefrin, and 114.4 per cent after inhalation. On Feb. 11, 1953, twenty-nine months after institution of hormone therapy, and while still taking HP Acthar gel, his maximum breathing capacity before Vaponefrin was 113.7 per cent of normal, and 122.1 per cent after Vaponefrin. These results were duplicated while this patient was on cortisone.

This man, with chronic pulmonary emphysema, who had been declared unfit for work and was considered to be a "pulmonary cripple," was restored to normal respiratory function after hormone therapy.

While it is improper to assume a patient cannot be improved until hormone therapy is tried, in general if the maximum breathing capacity before Vaponefrin is very low and that after Vaponefrin is still abnormally low, the clinical results with the hormones are not likely to be impressive. Patient 54, a man aged 53 years, with asthma for ten years, had been studied in the Cardio-Pulmonary Laboratory since November, 1950. His maximum breathing capacity ranged between 34 and 39 liters per minute, which was about 33⅓ per cent of the normal for him. He had always responded with Vaponefrin; the maximum breathing capacity increased after its administration to 52 liters per minute which, however, is low for adequate ventilatory function. The best maximum breathing capacity spontaneously obtained in this patient was 54 liters per minute, while receiving HP Acthar gel therapy in November, 1950. Clinically, this patient always had dyspnea. HP Acthar gel was eventually discontinued because of accumulation of edema. He died in May, 1953; autopsy revealed chronic bronchiectasis, emphysema, pulmonary fibrosis, cor pulmonale, and chronic passive congestion of liver, spleen, and kidneys.

Our conclusions concerning the usefulness of maximum breathing capacity determinations may be stated briefly. In the first place, a low maximum breathing capacity does not mean irreversible damage to pulmonary function if it is increased to 70 per cent or more of the predicted normal value after Vaponefrin and, by the same token, such a patient may be restored to functional comfort with HP Acthar gel or cortisone. Intercurrent pulmonary infection and exposure to inhalants to which the patient is sensitive may cause marked reduction in the maximum breathing capacity and in the response to Vaponefrin and hormones. Finally, from studies of respiratory function, it would seem that the progress of pulmonary cardiac disease may be delayed in many cases of intractable asthma by proper use of HP Acthar gel or cortisone.

DOSAGE OF HP ACTHAR GEL AND CORTISONE

Initial dosage of the hormones varied with the severity of the asthmatic state. The initial dosage of HP Acthar gel has been 40 units every four hours for the first twenty-four hours. As improvement appeared, the interval was increased to six hours. Usually within a week the patients were doing well on 40 units every twelve hours. From then on, depending on the progress, the dosage was lowered to 40 units a day for several days, then 40 units every other day, and gradually the point of minimum maintenance dosage was reached or the hormone discontinued completely. If, at the start of hormone therapy, the asthma is of the intractable type, but the patient is ambulatory and in no acute distress, the initial dosage may be 40 units of HP Acthar gel every day for a few days and as improvement occurs the hormone is given every second day and gradually reduced to the point of minimum maintenance dosage, usually 20 to 40 units two to three times a week.

With cortisone in severe status asthmaticus, the dosage usually effective is 100 mg. every four hours for the first twenty-four hours, every six hours the second twenty-four hours. Then 100 mg. is given every eight hours and within a week the patient is usually doing well with 25 mg. three to four times a day. From then on, depending upon the progress of the patient, the dosage is gradually cut to the amount necessary to keep the patient comfortable or the hormone is discontinued completely. For the ambulatory asthmatic, intractable to usual therapy, the initial dose of cortisone is usually 100 mg. a day and the dose regulated from this point. Many of the intractable cases do well with 50 to 75 mg. a day over long periods. Some will need only 37.5 mg. or 25 mg. a day. In our experience the most important point in maintenance therapy is to find the minimum amount of the hormone which will keep the patient comfortable. Larger amounts than this result in excessive weight gain, and tendency to edema and the moon face of the Cushing syndrome, without any greater relief to the asthmatic state.

ALTERNATION OF HP ACTHAR GEL AND CORTISONE

Twenty-four patients in this series had cortisone at one time and HP Acthar gel at another. Thirteen of these patients were started on hormone therapy using cortisone, and eleven first received HP Acthar gel. Whether the patient was started on cortisone or on HP Acthar gel seemed to make no difference in the clinical response to the other hormone given successively. In general, the patients had no particular dislike or preference for one hormone.

Patient 11 started on cortisone and then was given HP Acthar gel, of which she took 1,500 units during one month. She experienced swelling, soreness, and itching at the site of injection and had generalized hives. Naturally, she preferred cortisone. Another patient (No. 26) took cortisone from Jan. 10, 1951, to Jan. 10, 1952, with beneficial results. At that time his medication was changed to HP Acthar gel and he has continued receiving two to three injections a week with satisfactory result. Still another patient (No. 6) took HP Acthar gel from Oct. 12, 1951, to March 18, 1953. He was then continued on cortisone and did well. Patient 23 started on HP Acthar gel on Sept. 17, 1950, and

took it for ten days. He experienced great relief. He then changed to cortisone until Nov. 15, 1950, at which time he discontinued medication. A week later he was admitted in severe status asthmaticus to the New York Hospital and was given HP Acthar gel with good response. He took cortisone from Jan. 3, 1951, to Feb. 4, 1952, making good progress. He was then shifted to HP Acthar gel and his progress has been maintained. However, if either hormone is stopped his asthma will recur.

We have employed alternation of dosage in these cases, first, because we felt that prolonged stimulation of the adrenals with HP Acthar gel might cause irreversible changes and that prolonged use of cortisone might cause atrophy. Second, we wanted to find out whether therapy with one hormone might prevent a satisfactory reaction when a shift was made to the other. So far, we have found no cases where this has occurred, nor does initiation of therapy with one hormone alter the response when the shift is made. Our practice in shifting from one to the other has been, in certain cases, to gradually reduce the dosage of the previous hormone over a period of two weeks while the change is made to the other. This may be particularly important when changing from cortisone to HP Acthar gel, due to the lag in the Thorne test previously described. However, other cases have been shifted abruptly from one hormone to the other and we are unable to state from our clinical data that there is any basis for a gradual shift except the theoretical considerations mentioned.

GENERAL MANAGEMENT

Our patients are advised to avoid salt, are given potassium chloride or potassium iodide, and are impressed that intercurrent infection manifesting itself by fever or more purulent bronchial expectoration is a danger sign that requires the concomitant use of antibiotics. The recognition of intercurrent infection and the administration of suitable antibiotics make the difference between life and death in these patients. The urine is examined about one time a month for sugar, which is very rarely found. The blood pressure is taken regularly, but seemingly has not been influenced in patients taking the minimum maintenance dosage. We recommend careful determination of sensitivities by skin testing and appropriate immunization and elimination. Foci of infection in the upper respiratory tract are searched for and, where indicated, surgical removal is done, taking precautions mentioned earlier in the article. There is no question in our minds that hormones may be terminated or greatly reduced in patients where the usual allergic methods are followed concurrently with hormone therapy.

RÉSUMÉ AND DISCUSSION

In this study HP Acthar gel or cortisone treatment was given to sixty patients with asthma. Only eight (13 per cent) have either failed to respond clinically or were unable to take the hormones because of complications. Two patients with long standing asthma on small initial dosage failed to improve rapidly and discontinued treatment. Three patients had unsatisfactory results because they did not cooperate as regards either their general management or proper use of the hormones. In addition, treatment with hormones was

abandoned in three instances because of complications. In one case, HP Acthar gel was discontinued because the patient with advanced cardiac pulmonary disease experienced an increase of his edema. The two other patients with complications discontinued medication with cortisone because one of them developed depression and the other, with Raynaud's syndrome, developed peripheral vascular thrombi.

Thus, hormone therapy in asthma would appear to be a safe and effective method of obtaining symptomatic relief, even if given over periods of time which may be as long as, or even longer than, those reported.[1-4] It is essential to use proper precautions in selecting patients and to keep them under observation. It is also necessary to prevent sodium retention or loss of potassium. In surgical patients special management, as established by Salessa and his associates,[7] is necessary. The minimum doses of the hormones needed to keep the patient comfortable should be used, and factors underlying the asthmatic condition should be treated; this includes therapy directed against detectable hypersensitivities, infections (both acute and chronic), and other organic or psychogenic conditions.

In long-continued therapy, alternation or combination of parenteral administration of HP Acthar gel and oral cortisone is rational and effective. Whether the patient was started on cortisone or HP Acthar gel seemed to make no difference in the clinical response to the other hormone when given.

Eosinophil counts following the administration of HP Acthal gel has indicated a lag in adrenal function following cortisone therapy. On the other hand, the eosinophil count always indicated some degree of reactivation of the adrenal within two weeks after cortisone was discontinued.

Respiratory function tests are a useful guide to hormone therapy, but clinical improvement is more significant. In several cases, judging from the results of respiratory function tests, the progress of cardiac-pulmonary disease was apparently delayed through the use of HP Acthar gel or cortisone.

During the use of the hormones, awareness of the occurrence of intercurrent infection is most important and the use of antibiotics in this connection together with the hormones may be a lifesaving measure.

In general, it may be stated that the longer hormone therapy is continued, the smaller the amount of the drug that is required, if the factors leading to the asthma are progressively treated and managed.

SUMMARY

Sixty patients with asthma were treated with cortisone or HP Acthar gel, alone or in combination. Satisfactory results were obtained in fifty-two cases (87 per cent). Thirty patients continued to require maintenance of the hormones following institution of this form of therapy.

The largest amounts given to individual patients were 62 grams of cortisone and 7,780 units of HP Acthar gel, respectively. The longest continuous treatment given to any single patient extended over a period of thirty-nine months. Combined or alternating treatment with the two hormones was used successfully in twenty-four instances.

Complications were rarely encountered. Seven patients included in this study died, but only two of them were receiving hormones, and those in small doses at the time of death.

Usefulness and limitations of respiratory function tests and of the Thorne test in guiding hormone therapy are discussed.

Need of over-all management of patients with asthma is emphasized; it is concluded that, with proper precautions, HP Acthar gel and cortisone may be used to relieve acute attacks, and also may be employed over prolonged periods of time with resulting marked clinical relief to the asthmatic state.

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BRONCHIAL ASTHMA UNRELATED TO POSITIVE SKIN REACTIONS

A Psychosomatic Study

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IT IS frequently noted in allergy practice that patients with well-marked skin reactions are inconsistent in their clinical response.[1] The reasons for this are not clear. Having studied a patient in whom the inconsistency was particularly striking, we are presenting our observations and offering a possible explanation.

The patient, a boy 3 years and 8 months of age, was the second of three siblings. His asthma had started a year before he came to us. Between attacks he had some nasal obstruction. Since infancy he had shown a mild aversion to eggs.

On examination, he was found to be frail-looking and timid. The chest was typically asthmatic.

Skin tests resulted in 3- to 4-plus positive reactions to practically all the pollens, many positive reactions to other inhalant substances and to numerous foods, that to egg white being most marked.

At no time were restrictions placed on diet or physical environment, and no specific allergy treatment was administered.

During the period of observation, from February, 1953, to Dec. 31, 1953, twelve episodes of wheezing occurred in all. Only two of these were protracted; one in June, 1953, and the other in December, 1953.

The June episode lasted from the eighteenth to the twenty-fourth. According to Targow[2] the peak of the grass pollinating season in the vicinity of Los Angeles is in May, averaging 124 pollen grains per 2 sq. cm. of slide surface daily. In June, 1953, during the period of the attack, they averaged 1.8 grains per day.[3] That is, the attack of asthma, despite the marked skin sensitivity to grass pollens, occurred well after the peak of the grass pollinating season.

Climatological data[4] showed no significant contrasts.

Moreover, according to the diet diary, there was no deviation in the kinds of food eaten, including the reacting foods, milk, avocado, banana, pineapple, and egg in some form daily; although he ate no whole egg as he did on July 7, thirteen days after the attack.

No change could be noted in the immediate environment.

The December episode lasted from the fifteenth through the twenty-first, coinciding in time of year with that of a similar, more serious attack in De-

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cember, 1952. Both years during this period, the average pollen count was 0.7 grain.[3] Again, no variations in diet had occurred.

In short, there was no apparent correlation between the attacks of asthma and exposure to the various allergens which gave positive skin reactions, nor to weather or other environmental physical variables.

The thesis is therefore held, as by Walzer[5] and Hill,[6] that the positive skin reactions merely represented an immunologic response to previous exposures to the allergens.

Meanwhile, from March 18 through Dec. 31, 1953, the patient had 32 psychotherapeutic play sessions (DWB), the father 73 psychoanalytically oriented psychotherapy sessions (DWB), and the mother 95 similar sessions (HM). This gave the opportunity to observe minutely the emotional interplay within the family and thus to gather situational and life-stress data associated with each of the 12 attacks of asthma.

From the mass of data, a characteristic pattern, previously described by Miller and Baruch,[7] became evident; namely, that wheezing coincided with the inability on the part of the patient to openly express the anger which accrued when he felt deprived of or threatened by the loss of parental affection.

The following descriptions and verbatim quotations indicate the emotional climate in which the patient was living.

The mother was an intelligent, dominating woman. It was extremely difficult for her to express her feelings, either of anger or of warmth. Her psychotherapeutic sessions usually began with complaints about her children's health, diet, and behavior. Despite this, her hostility came out in statements such as the following:

"I get so mad I think maybe I don't love my children. They drive me to distraction. I never had a real deep desire for a child. I'd rather dig a ditch than feed them."

The father was more open than the mother. Although he had a tendency to flare up in anger, he also was able to be openly warm. He was a dependent person, eager to please his wife. Prior to his psychotherapy, he had accepted the role which she had imposed on him as a punitive disciplinarian. After some psychotherapy, he saw that the children needed more affection. Since his office was in his home, he could readily give this, even to assuming the children's physical care. This his wife willingly relinquished and the children readily accepted. To quote him: "The children want me. I guess I'm like their mother now."

Of his wife, he said: "She doesn't ever want to stay with the children. She can't even stand feeding them."

That the patient also felt these attitudes was evident from his psychotherapeutic play sessions. In the child's third session, for instance, he sat on his therapist's lap and wanted her to do nothing but feed him. He ate 26 chocolate candies. Every once in a while he looked up shyly and said, "I like you, you're my feeding mommy. You're not my poison mommy."

When he drew a picture of his family, he left himself out, shook his head, and finally said, wheezing, "In that family the mother doesn't want a boy."

He referred in great fear to the "poison lady" who would feed him poison and would drown him in the tub.

His resultant anger became apparent, although, as is characteristic with asthmatics, he would, on many occasions, block and cover it. Such blocking was found definitely associated with the attacks. To cite an example:

At 9:30 one morning the father phoned the therapist. He reported that he and his wife had gone out the previous evening. When they returned at 10 P.M. they found Donald wheezing in his sleep. He awoke and said, "Nobody loves me," and continued wheezing, whimpering, and complaining through the night; however, he expressed no anger. After the phone call, the father, following the therapist's suggestion to him, said: "You feel nobody loves you, and that naturally makes you mad."

All of a sudden the child yelled, "I want to kill you. I'd like to lasso you and choke you around your neck and I'd like to cut open mommy's stomach and hit her and everybody with a baseball bat."

Forty-five minutes later, the father phoned that the wheezing had stopped.

The relatively severe attack of asthma in December, 1953, when examined in the light of the more severe attack in December, 1952, lends itself particularly well in tracing the life situation and the emotional stresses to their ultimate expression in somatic symptoms. In both years, it will be remembered, the exposure to pollen was minimal or absent. Food and physical environmental factors[4] presented nothing atypical. The life situation, however, included a special and specific circumstance; namely, the coincidence of the younger sister's birthday with Christmas Day. It now became apparent that in the patient this set off anger because of the fear that his parents would turn their affection from him to his younger sister.

Whereas, in 1952, he had not expressed his resentment and had apparently been happy about his sister's birthday, in 1953 he was more open about his real feelings. He said, "Betty's a bad girl. I'll make her all dead." To his father he said, "I want to throw the Ping-pong paddle at you and I want to spit on you. I don't like you because you like Sister." At this, the father said to him, "You can't do those things, but you can tell me all about them and I won't get mad at you." The patient remained free of asthma as he had been for the previous twelve weeks.

On December 14, however, the father, in a fit of anger at his wife, absented himself from home, leaving her to care for the children. In the evening, he returned in pain, having hurt his leg. He crossly refused to bathe the children. The mother then bathed Donald, a duty she had avoided for six months. The boy became very quiet, appeared frightened, began to whine and complain that nobody loved him. The father scolded and sent the child to bed.

At 5 o'clock the next morning Donald awoke wheezing. The father tried to help him bring out his anger. However, frightened by the father's desertion of him as a good-mother figure and his exposure to a threatening mother,

the child continued to block. For six days, on account of the father's injury, the mother went on bathing the child. For six days he did not express any anger. During this period wheezing persisted.

On the sixth day, December 21, the child came to his therapy session wheezing, very frightened, and withdrawn. Finally, he managed to say, "Do you know, Dorothy, I'm scared. My Daddy's no fair. He had Betty on Christmas Day. He's no fair. He and my mommy give Betty two times my presents." He then got out the water gun and the doll family, saying, "I'll kill them all, every one. Then I can go home and live with you. I don't like the poison lady. I don't like her at all. She wants to drown me." He continued to shoot the "poison lady" and to drown the whole family in a bowl. By the end of the session, the wheezing had disappeared.

Subsequently, the father and the mother, in their sessions, were able to bring out their own anger, becoming acceptant enough for the child once again to feel safe in expressing his anger. He took out his play gun, for instance, and played that he was shooting his parents, saying he wished he had real bullets. He got a pretty little box, filled it with mud and put it under the tree as his present for Betty. Continuing thus, he went through the Christmas birthday without wheezing, remaining clear up to this writing (Jan. 4, 1954).

This episode was relatively short and mild in contrast to that of the preceding year when, as the father said, "We made him be nice all the time."

The sequence of events in the Christmas episode was also evident in all the other eleven attacks. In each instance, it was possible to trace the basis for the anger, to observe its evident blocking, and to observe the relief of asthma when hostility was brought out.

Although we have been concerned in this report primarily with the relationship between the emotions and bronchial asthma, it is evident that treatment has been directed to the patient as a whole and not just to a single symptom. It is not surprising, therefore, that not only was asthma relieved, but that there was great gain in appetite, a loss of diffidence, and an increase in aggressiveness, and great improvement in the relationships with the other members of the family.

SUMMARY AND CONCLUSIONS

A boy of 3 years, having recurrent attacks of bronchial asthma, was found to have numerous positive skin reactions to food and inhalant allergens.

Study of pollen counts, the weather, the diet, and the immediate physical environment showed no correlation between asthmatic attacks and exposure to the reacting allergens.

The thesis is advanced that the positive skin reactions were an immunologic response to previous exposures to allergens.

Play psychotherapy with the child and psychoanalytically oriented psychotherapy with each parent were instituted.

The data from these sources indicated that the emotional interplay could be correlated with the asthmatic attacks of the child.

Evidence is given that indicates that the emotional pattern consisted of a feeling on the part of the child that he was not loved and that when his anger at his parents on this account was blocked from expression by his fear, asthma would result.

In contrast, when his anger was expressed and he was made to feel secure in his expression of it, his asthma could be aborted or, when present, cleared.

It is concluded that, in this patient, emotional problems were expressed somatically as clinical allergy which functioned within a pattern set by his allergic constitution quite independently of his exposure to reacting allergens.

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STUDIES IN SENSITIZATION AS APPLIED TO SKIN TESTING PROCEDURES

II. Influence of Allergen Contact

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ALLERGISTS long have recognized the important role which contact with allergens plays in the mechanism and production of clinical sensitization. Adequate discussion of this subject, particularly as related to hereditary predisposition, is to be found in all monographs or books on allergy.[1] Grove probably was the first to demonstrate this in some unpublished observations reported in 1931 by Coca.[2] She tested thirty-six timothy hay fever subjects residing in Berlin, where there is no ragweed, with a strong ragweed pollen extract. All reactions were negative except in one person who had spent some time in the United States and was the only one to have had contact with ragweed pollen. These observations subsequently were substantiated by others and serve to explain the common finding of the "nonclinical" positive skin test reactions. Despite the general acceptance of the role of contact in sensitization, little is known about the amount of contact required to induce sensitization, which on the one hand may be limited to the skin and indicated only by a positive skin test reaction, or may involve other tissues as well and be responsible for associated clinical symptoms. Many believe that it depends largely upon the sensitizing ability of the offending allergen as well as upon the degree of maturity of the sensitized cells, the latter being influenced perhaps by hereditary predisposition. As a consequence of these influences, when sufficient antigenic stimulation is provided by adequate contact with the specific excitant, evidence of hypersensitiveness will appear. The development of this sensitivity may be hastened either by contact with an excessive amount of the allergens or by nonspecific influences.

Adequate clinical and experimental proof of these ideas already has been offered by various investigators, who have shown, for example, that sensitization can be induced artificially in any person (atopic or nonatopic) by many agents if the contact is adequate and sufficiently prolonged. Thus, horse serum will sensitize all individuals if injected in sufficient amounts and especially if given intravenously.[3] Poison ivy also will sensitize all individuals if the contact is adequate. On the other hand, as shown by Brunner,[5] atopic individuals could not be sensitized by known powerful atopens (for example, egg white, rabbit epithelium, or orris root), nor do nonatopic individuals exposed to large amounts of pollen or injected with its extract develop hay fever.

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Although allergen contact admittedly is of paramount importance in the development of clinical sensitization, the attending allergist, nevertheless, usually is more concerned with determining the clinical or nonclinical nature of the positive skin test reaction than with attempts at determining the manner in which the sensitization developed, especially in its initial phase or preclinical period. In order to bridge this gap, the present series of studies has been undertaken. These studies have as their purpose the investigation of the following criteria of sensitization: the susceptibility of the individual; the nature of the sensitizing substances, as well as the mode and duration of contact; and, finally, whether the process resulting in a positive skin test reaction differs from that bringing about clinical symptoms. In our first article,[7] we reported the results of investigations seeking to answer the question as to whether or not positive skin tests change under the influence of such factors as avoidance or specific treatment. The present report concerns itself with our studies of the role and importance of contact in relation to sensitization. The first portion of the report concerns itself with studies done in patients allergic to horse serum; the second portion with studies into sensitization with foods eaten often or infrequently.

A difficult problem for the allergist is the correct interpretation of "nonclinical" positive skin test reactions, those provoked by prior contact with antigens which sensitize the skin but without concomitant clinical symptoms. A striking example of this is seen in patients with clinical ragweed hay fever who have positive skin test reactions to grass pollens but no clinical evidence of grass pollinosis. While such nonclinical positive skin reactions are the result of previous contact with grass pollens, the extent of contact necessary to induce this skin sensitization is unknown, as well as that required to induce clinical sensitization. This process of sensitization applies not only to pollens, but also to foods with which the patient comes in contact early in life and which likewise could account for either clinical or nonclinical positive skin test reactions. Thus, Schloss[8] in 1920 showed that the ingestion of egg white by marantic infants induced sensitization indicated both by a positive skin test and circulating antibodies, but without associated clinical manifestations of allergy. Subsequent clinical experience has shown that food contact, especially in young children, may provoke temporary clinical symptoms; later they seem to acquire a tolerance and can eat the food again with impunity. If tested during the latter period, such patients still have a positive skin reaction, which now is the only evidence of their previous antigenic contact. Such a reaction may persist for long periods, even throughout the patient's life. This is true, for example, of children clinically allergic to milk or egg in infancy who later take these foods without difficulty, but in whom the positive skin reactions are proof of their former sensitizing contacts. Unless the allergist tries to obtain a detailed history of their clinical reactions to these foods in infancy or childhood, such nonclinical positives may be regarded as "nonspecific" or "irritating," with no attempt being made to investigate the reasons for their occurrence.

It seemed to us that the subject could be clarified if additional evidence was provided showing that contact with specific allergens may induce specific skin sensitization, regardless of the presence of clinical sensitization. Food and pollen allergens would seem to offer the logical approach to this problem; but, unfortunately, these allergens are so ubiquitous and varied as to raise valid objections. For example, it would be difficult to obtain a reliable history from such a patient as to the extent of contact which was responsible for the production of his positive skin test reaction. Even if this obstacle could be surmounted, it would be hard to overcome another objection, namely, that the sensitization was induced by exposure to homologous food antigens present in other foods. For example, a positive reaction to lobster could be explained by sensitization with a similar antigen present in shrimp or crab;[9] similarly with pollens, exposure to one type of ragweed would cause sensitization to other related types.

The same objection, it seemed to us, did not apply similarly to exposure to an antigen like horse serum. Except for patients who are sensitive to horse dander or who might have ingested horse meat, a patient's only possible exposure to horse serum is that which results from the injection of an immune agent or mixture containing horse serum. If sensitization ensues, it would be indicated by a positive skin test reaction to horse serum, but not all patients receiving horse serum are sensitized. Hooker[10] in 1924 and Tuft[11] in 1932 both showed that about 25 per cent of children who had received, at weekly intervals, the usual three immunizing injections of diphtheria toxin-antitoxin containing extremely minute amounts of horse serum later showed positive skin test reactions as indications of active sensitization. That this sensitization was not necessarily clinically important was substantiated first by Park[12] and subsequently by clinical experience which indicated that horse serum could be reinjected into some skin test positive patients without inducing a reaction. However, as shown by Tuft,[11] the incidence of serum reactions was greater in patients previously sensitized by toxin-antitoxin. Therefore, a positive skin test reaction to horse serum in a patient not sensitive to horse dander or horse meat is an indication of previous contact with horse serum, regardless of whether the sensitization is limited to the skin or involves other tissues as well. This should provide additional and incontrovertible evidence of the importance of allergen contact in sensitization, since such sensitization, with the rare exceptions already mentioned, could have resulted only from previous injection.

In an attempt to provide such evidence,* we reviewed the case histories of 528 clinic and private patients (most of whom were being looked over for other investigative purposes[7]) to determine the incidence of horse serum sensitization as indicated by a positive skin test reaction to our routine testing strength of horse serum (1-100). At the same time, the reaction to horse epithelium in our routine testing strength (0.001 mg. N per milliliter) likewise

*As described in our previous article,[7] all skin tests were done intracutaneously in the routine dilutions used in all our allergic patients. Every positive test, regardless of degree, was checked by a repeat test done later in order to determine the constancy of the reaction. Whenever comparisons were made, we were careful to compare the tests in the same concentrations. This was true not only for horse serum but also for foods, pollens, or other inhalants.

was recorded to see whether there might be an antigenic relationship. In all instances, and especially in those patients with positive reactions to horse serum, the history was reviewed for evidence of previous injection of horse serum or of clinical sensitivity to horse epithelium in an effort to substantiate the positive reactions. Patients usually do not offer information about previous injections unless specifically requested, since it has no particular bearing upon their allergic complaint. Consequently, many of our histories did not contain this information when the patient was first seen. However, after completion of the allergic studies, when the skin test results of all private patients were reviewed with the patient or, in the case of a child, with the parents, a special effort was made to get a history of previous injection of agents containing horse serum whenever a positive reaction to horse serum was obtained. In this way we often obtained in retrospect more definite information about previous injections of horse serum than was supplied by the original history.

TABLE I. RESULTS OF HORSE SERUM REACTIONS IN 528 CASES

	NUMBER	PER CENT	TOTAL NUMBER
Negative skin reactions to horse serum			458 (86.7%)
1. Negative history of previous injection			
a. Negative horse serum, negative horse epithelium	384	83.8	
b. Negative horse serum, positive horse epithelium	11	2.4	
2. Positive history of previous injection			
a. Negative horse serum, negative horse epithelium	60	13.1	
b. Negative horse serum, positive horse epithelium	3	0.7	
Positive skin reactions to horse serum			70 (13.3%)
1. Positive history of previous injection			
a. Positive horse serum, negative horse epithelium	40	57.1	
b. Positive horse serum, positive horse epithelium	17	24.3	
2. Negative history of previous injection			
a. Positive horse serum, positive horse epithelium	6	8.6	
b. Positive horse serum, negative horse epithelium	7	10.0	

The results of this investigation are summarized in Table I. It will be observed that of the total number of records reviewed, 458, or 86.7 per cent, had a negative test to horse serum. Of this negative-reacting group, most (384, or 83.8 per cent) gave a negative history of previous serum injection, whereas 60, or 13.1 per cent, had a positive history of previous injection and a negative skin test, indicating an absence of sensitization despite previous contact, which is in accordance with the previously mentioned experiences of Hooker[10] and of Tuft.[11] It is of interest in this connection that a small number (11, or 2.4 per cent) of the group with negative histories of previous injection had positive tests to horse epithelium but negative tests to horse serum; also, there were three additional patients with positive tests to horse epithelium who had negative reactions to horse serum, despite a positive history of previous horse serum injection. These findings indicate that, while patients sensitive to horse epithelium often also are sensitive to horse serum because of the presence of a common antigen,[13] this is not always true; therefore, patients may be sensitive to horse epithelium alone without being sensitive to horse serum, and on occasion even may be given a horse serum injection without resultant reaction.

Of more interest in relation to the present investigation was the review of the seventy patients showing positive reactions to horse serum (Table I). Of the seventy, forty-seven were positive to horse serum alone and twenty-three to both horse serum and horse epithelium. In the latter group, the positive reaction could be accounted for either by a previous serum injection (in seventeen) or by homologous antigenic relationship in the absence of a history of previous injection. The latter was true in six patients, five of whom had definite clinical evidence of sensitivity to horse epithelium (asthma or coryza, or both, after contact with horses). However, of the entire group of seventy with positive reactions to horse serum, only seven patients (10 per cent) with concomitant negative tests to horse epithelium failed to give a history of previous horse serum injection or at least were uncertain as to whether they ever had received a previous injection. It is possible, of course, that some of these might be ascribed to technical error in performing the skin test, but a more plausible explanation probably is the failure to get a complete history of previous serum injections. Since most of these patients were adults, they might have received toxin-antitoxin injections when they were young but did not remember it. That failure to obtain such a history does not exclude a possible previous horse serum injection was emphasized frequently when we reviewed the history either with the patient or with the parent (if the patient was a child) after a positive skin reaction to horse serum had been obtained. Often, when asked specifically about previous injections, they then would remember having received some type of serum injection, even though they did not consider it important enough to mention or had forgotten it when asked at the initial visit. This is nicely exemplified in the following two brief case reports:

CASE 1.—A 10-year-old boy had a positive reaction to horse serum. Upon asking the patient's mother whether he had ever received an injection, she immediately denied it. But at her next visit she stated that after thinking about it more she recalled that the patient was bitten by a dog some years previously and at that time was given "a serum injection against lockjaw," with no particular reaction. Obviously, had we taken the initial word of the mother, we would have considered the history of previous injection negative and thus would be unable to explain the positive horse serum reaction.

CASE 2.—A boy, 12 years of age, seen for hay fever, had a positive skin reaction to horse serum. When asked about it at his next clinic visit, he denied having received any previous serum injection. We instructed him to ask his parents when he got home. Upon his return he told us that his mother recalled that at the age of 3½ he had an accident to his thumb for which he received a "lockjaw injection."

In both these instances, as in others, if we had relied solely upon the initial history we could not have determined the reason for the horse serum sensitization indicated by the positive skin test. This perhaps also was true of the small number of patients in the group who had positive skin tests to horse serum but who were not too certain as to a previous serum injection. Nevertheless, despite these exceptions, the evidence obtained from a survey of this group of patients clearly indicates that a positive skin test to horse serum constitutes definite indication of previous sensitization by horse serum which could not be acquired in any other way except by previous antigen contact,

usually artificially induced. This is true regardless of whether the patient has a clinical sensitization and would develop symptoms upon serum reinjection (a clinical positive) or whether the sensitization is limited solely to the skin (nonclinical positive). Once the sensitization has been acquired, it may persist as a positive skin test reaction for varying lengths of time, either unchanged or in lesser degree. In one patient with a positive test to horse serum, there was an interval of twenty-five years between the time of injection and the positive skin test, indicating that such sensitization can persist for long periods.

If this evidence of previous contact as the primary factor in the production of sensitization is true for horse serum, it should apply also to sensitization provoked by food and pollen antigens. Unfortunately, as previously mentioned, in the latter instances it is much more difficult to obtain adequate proof of previous allergenic contact or its extent, except at times from clinical history or experience. Thus, for example, it is not unusual in older children to obtain positive skin reactions to such foods as milk or orange which they can eat without provoking any clinical symptoms. However, detailed inquiry into the past histories of such patients often reveals that during infancy or early childhood, they experienced considerable difficulty when taking milk or orange; but, later, either after abstinence or at times after continued ingestion, they apparently were able to overcome the clinical sensitivity and could eat the food in question again without untoward effects. Nevertheless, such patients still had a positive skin test reaction long afterward as concrete evidence of their previous clinical sensitization. If this is true of milk or orange, it also must apply to other foods but, since one must depend entirely upon clinical findings in these instances, it is difficult to secure good objective evidence in support of the contact factor. During the course of our investigations into changes in retest reactions, already reported in a previous article,[7] we obtained additional evidence of the influence of allergenic contact which we are now reporting. In our first study we were concerned primarily with comparing the retest results with that of the original test after the lapse of varying time intervals. It was soon evident that certain patients showed newly acquired sensitivities, as indicated by positive skin reactions to foods* to which they formerly were skin test negative. Thus, of forty-eight patients in whom the retest results were compared and in whom the food histories were reliable, fifteen patients had a total of thirty-three newly acquired, but definite, positive skin reactions to various foods, some patients having positive reactions to more than one food. Upon reviewing the histories of these patients, it was found that in twenty-eight instances out of the total of thirty-three, the sensitization was to foods frequently eaten by the patient, whereas in the remaining five the sensitization existed to foods which were uncommonly eaten. Furthermore, in thirty of the thirty-three instances of newly acquired positive reactions, we elicited the history that this was a new contact with these foods, since the patient did not recall having eaten them previously. In addition, further review of our data indicated that the longer the duration of

*See previous footnote.

contact with the food, the greater was the chance of sensitization, since a larger number of patients showed positive reactions when retested five or more years after the original than if retested before five years. In certain patients, too, after review of their food histories, we showed that excessive antigenic contact with certain foods seemed to induce sensitization at least as indicated by a positive skin test reaction.

This evidence strongly suggested that both the type and the duration of allergenic contact were important factors in the acquisition of sensitization to foods so that the greater the extent of contact, the more likely was sensitization to result; also that sensitization is more likely with foods eaten often than with those which were not eaten so frequently. The latter evidence seemed contrary to the commonly held viewpoint that food sensitization depended largely upon the antigenic nature of the food. Therefore, in order to obtain more definite evidence, we attempted to determine from the review of a substantial number of our skin test records the relative incidence of positive reactions to such commonly eaten foods as banana, chicken, chocolate, cornmeal, egg, flounder, lobster, milk, orange, oyster, green pea, peanut, strawberry, tomato, and wheat in comparison with the incidence of positive reactions to such uncommonly eaten foods as blackberry, chestnut, goose, green pepper, sweet potato, pumpkin, and rhubarb. In all instances we included only those positive reactions which were slight to marked in degree and which had been rechecked by repeated tests in the same concentrations as the original test. Whenever new positive reactions were obtained, we reviewed the history with the patient to ascertain whether these foods were eaten frequently, only occasionally, or not at all during the period between the original or initial test and the retest.

The data obtained in this review are summarized in Table II. Inspection of the latter reveals at once a significantly higher incidence* of positive reac-

TABLE II. POSITIVE REACTIONS TO COMMONLY AND UNCOMMONLY EATEN FOODS

FOOD ALLERGEN (COMMON FOODS)	TOTAL NUMBER OF PATIENTS	PER CENT TOTAL* POSITIVES	FOOD ALLERGEN (UNCOMMON FOODS)	TOTAL NUMBER OF PATIENTS	PER CENT TOTAL* POSITIVES
Banana	670	21.6	Blackberry	657	7.5
Chicken	663	6.2	Chestnut	647	4.5
Chocolate	673	36.5	Goose	640	2.9
Corn meal	672	20.2	Pepper (green)	641	6.2
Egg	673	7.6	Potato (sweet)	661	7.3
Flounder	670	4.6	Pumpkin	644	7.3
Lobster	646	13.0	Rhubarb	651	7.4
Milk	673	10.5			
Orange	671	14.9			
Oyster	652	9.0			
Pea (green)	672	41.4			
Peanut	657	12.4			
Strawberry	652	13.8			
Tomato	675	60.7			
Wheat	670	13.4			

*P = <.0001 (highly significant).

*The figures in this table were subjected to statistical analysis by the X^2 formula. We are indebted for this and subsequent analyses to Dr. Hugh Hamilton, Professor of Psychology, Temple University, Philadelphia, Pennsylvania.

tions to most of the foods commonly eaten (left-hand column) than to those uncommonly eaten (right-hand column). Some percentages seemed unusually high, such as those for tomato, green pea, and chocolate, three very commonly eaten foods. But this is true for most of the commonly eaten foods which individually showed significantly higher figures statistically ($P = <.0001$) than the foods eaten infrequently. At first glance one might attempt to explain this difference upon the basis that the commonly eaten foods contained larger amounts of active allergens which are more easily capable of inducing sensitization. It seemed hard to believe, however, that substances like goose or chestnut which are relatively high in protein would be less allergenic than chicken or peanut, or that strawberry is more allergenic than blackberry. When the percentages for these particular foods are compared, as in Table III, it immediately is apparent that this is not a valid explanation for this difference, since there was a definite and statistically significant difference between the incidence of positive reactions to chicken as compared with goose, to peanut as contrasted with chestnut, or to strawberry compared with blackberry. While the amount of allergen present in a food undoubtedly is a factor, it becomes important only if the contact factor also is present. This accounts for the difference between the incidence in the two groups.

TABLE III. COMPARISON BETWEEN COMMON AND UNCOMMON FOODS

FOOD ALLERGEN	TOTAL NUMBER PATIENTS AND/OR TESTS	TOTAL POSITIVES (PER CENT)
Chicken	663	6.2*
Goose	640	2.9
Peanut	657	12.4*
Chestnut	647	4.5
Strawberry	652	13.8*
Blackberry	657	7.5

*All statistically significant ($P = <.01$).

It might be said in objection to the figures obtained in Table II, especially in relation to the high percentages of positive reactions to such foods as tomato, green pea, or chocolate, that we were dealing with extracts which gave nonspecific positive reactions because of either faulty preparations or the presence of irritating materials. If this was true of these extracts, then one would expect high percentages in similar tests conducted in a control nonallergic group. To determine this, we tested a group of 100 nonatopic individuals, all students between the ages of 21 and 30, in whom we could get no history of past or present atopic disease (asthma, hay fever, migraine, or eczema). All were tested with extracts of those foods (common and uncommon) which we had used to test the allergic patients. Furthermore, in order to make the comparison more accurate, we selected at random a group of 100 allergic patients whose ages also were between 21 and 30. We then determined the incidence of positive skin tests* in these two comparable groups for both groups of foods. The results are shown in Table IV.

*All done intracutaneously in the same comparable dilutions used routinely in testing our allergic patients.

TABLE IV. FOOD TESTS IN ALLERGICS AND CONTROLS*

FOOD ALLERGEN† (COMMON FOODS)	CONTROL (NONATOPIC)	ALLERGIC	FOOD ALLERGEN§ (UNCOMMON FOODS)	CONTROL (NONATOPIC)	ALLERGIC
Banana	4	21	Blackberry	5	10
Chicken	2	4	Chestnut	2	5
Chocolate	13	33	Goose	2	4
Corn meal	12	22	Pepper (green)	0	6
Egg	2	6	Potato (sweet)	3	9
Flounder	2	3	Pumpkin	4	9
Lobster	11	15	Rhubarb	7	7
Milk	1	10			
Orange	3	17			
Oyster	4	11			
Pea (green)	5	44			
Peanut	1	11			
Strawberry	14	14			
Tomato	7	60			
Wheat	10	14			

*Done on 100 patients in each group, all aged 21 to 30 years.

†For comparison between common foods in allergics and controls. P = <.001 (highly significant).

§For comparison between uncommon foods in allergics and controls. P = <.53 (not significant).

Inspection of this table at once shows rather strikingly that the incidence of positive reactions to the commonly eaten foods is definitely and significantly higher in the allergic than in the control group. This is especially true of those foods showing a high incidence of positives, notably tomato, green pea, and chocolate. For example, the incidence for tomato in the allergic group was 60 per cent, compared with 7 per cent for the control group; for green pea it was 44 per cent, compared with 5 per cent; and for chocolate it was 33 per cent, compared with 13 per cent in the control group. This striking difference indicates that the high incidence could not be due to the presence of nonspecific or irritating material, but that allergic individuals apparently are more readily sensitized by foods which they eat commonly than are nonallergic persons.

On the other hand, when we compare the incidence of positive skin test reactions in allergic patients to foods uncommonly eaten with that of the control group, it is immediately apparent that, while the incidence in the former is somewhat higher than in the control, the difference between the two is not sufficient to be statistically significant (P = <.53); or to put it another way, foods infrequently eaten are not likely to induce any greater incidence of skin sensitization (as indicated by a positive skin test reaction) in the allergic patient than in the nonallergic or control group. The inference to be drawn from these observations is that all individuals, especially allergics, are sensitized more readily by foods which they eat often and in large amounts than by those which they eat infrequently.

While this has no particular bearing upon the question of allergen contact, which is the subject of the present report, an interesting sidelight of our results is the relatively low incidence of positive reactions to most of the food allergens in the control group. This at first would seem contrary to the older

observations, particularly those of Grow and Herman,[15] who in 1935 reported much higher percentages in a group of 150 normal persons tested with thirteen common allergens. However, if the doubtful positive reactions were not included in their estimation of percentages and if the inhalants were excluded from consideration, their figures would be lower and would compare favorably with our figures as to the incidence of specific positive reactions to food allergens in normal persons.

COMMENT

While the results of these reported studies verify the clinical observation that contact with allergenic agents is extremely important in the induction of sensitization, they also show that this is more true of allergic or atopic individuals than of normal or nonallergic control groups. Thus, sensitization more frequently followed exposure to food allergens which are eaten frequently and in larger amounts than exposure to those eaten only occasionally and in small amounts. This was substantiated by our finding that there was a higher incidence of positive skin test reactions to foods frequently eaten (tomatoes, chocolate, or peas) than to those which are infrequently eaten (goose, chestnut, green pepper, and the like). The factor of allergenic contact in these instances probably is not the sole one. Undoubtedly, the amount of active allergen present in a particular food also must be important but, as we showed in our studies, it required a combination of the two factors, namely, a potent sensitizing active principle as well as extensive allergenic contact, perhaps aided by hereditary predisposition to bring about definite sensitization. The latter may be limited to the skin only and manifest itself only as a positive skin test (a nonclinical positive) or it may involve other tissues as well and give rise to clinical reactions. While nonclinical positives may indicate past or future (potential) clinical sensitization, additional proof that they indicate past sensitization was furnished by our studies of patients with positive reactions to horse serum, in which we showed that such reactions almost always resulted from previous exposure to horse serum regardless of the route. Since the skin is such an excellent memory organ, it retains this evidence for years, perhaps for the remainder of the patient's life. Thus, in one of the patients, the positive reaction to horse serum remained for twenty-five years after the initial sensitization, despite the lack of continued antigenic contact.

Just what it is that converts a nonclinical positive into a clinical one, associated with clinical symptoms, still remains to be determined. Unusual or excessive antigenic stimulation undoubtedly is a factor. This was true, for example, in some of our patients who were sensitized by the ingestion of larger amounts of certain foods (for instance, milk) than they previously had taken. Under those circumstances, it is not unlikely that excessive antigenic exposure may convert a previously existing nonclinical sensitization, localized primarily to the skin, into one which also affects other tissues and manifests itself in clinical symptoms.

From a practical clinical standpoint, a significant lesson to be learned from these observations, and one which may be important in prophylactic

treatment, is that allergic patients, especially children, with significant positive reactions to commonly eaten foods should be warned against eating such foods in large amounts as a preventive measure against possible future clinical sensitization.

Finally, the results of these studies also suggest another point which might be applied profitably in clinical practice. Since the incidence of positive reactions to foods uncommonly eaten has been shown to be rather low and not much different from that found in the nonallergic controls, it would seem unwise to continue testing patients with foods which they eat infrequently and/or in small amounts, since the chance of discovering the existence of such sensitization, at least in our experience, seems to be remote. Elimination of tests to foods uncommonly eaten would shorten our testing procedures and serve to streamline our test lists. This, according to information obtained from our questionnaire data,[7] is something desired by allergists, for it would serve to increase the value of and respect for our testing methods.

CONCLUSIONS

From the results of our observations and studies, the following conclusions seem warranted:

1. Allergenic contact is extremely important in the induction of sensitization, more so in atopic persons than in normal persons.

2. Sensitization, at least as indicated by positive skin reactions, is more frequent after exposure to foods eaten frequently and in large amounts than to those infrequently ingested.

3. Sensitization, once induced, may persist for extremely long periods of time as exemplified by sensitization to horse serum. Since we have demonstrated that a positive skin test reaction to horse serum constitutes definite and incontrovertible evidence of previous contact with horse serum, it should apply equally to other allergens, inhalant or food, and thus explain the existence of many positive reactions which appear to have little clinical significance (nonclinical positives).

4. Allergic patients, especially children, with positive tests to commonly eaten foods should be advised against eating these in large amounts, since excessive antigen contact may convert a nonclinical sensitization into a clinical one.

5. Since the incidence of positive skin test reactions to foods infrequently eaten is quite low, their elimination from the routine skin test lists would seem advisable, as it would shorten the testing procedure materially.

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CEREBROSPINAL RHINORRHEA SIMULATING ALLERGIC RHINITIS

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CONSIDER the case of a patient suffering from serous nasal discharge of recent onset coinciding with a change in living quarters. The environmental and time relationships suggest an allergic etiology. Accordingly, the patient is likely to be referred to the Allergy Clinic. While we have come to regard the history as the major diagnostic tool, the present report emphasizes that even an apparently clear-cut correlation between cause and effect may be misleading unless confirmatory evidence is obtained. Several causes for rhinorrhea are well known, but its differential diagnosis is a problem faced every day by the allergist. In the practice of allergy, it is frequently necessary to eliminate simple vasomotor instability and infections which simulate or obscure allergic conditions. This report is offered as a reminder that cerebrospinal rhinorrhea, although quite rare, can produce apparent "rhinitis."

In the case to be described, not only did the onset of symptoms coincide with a change in the patient's living quarters, but, in addition, they also occurred during the spring and summer pollen seasons. These highly suggestive events supported the likelihood of allergy, and delayed the proper diagnosis.

CASE REPORT

The patient, a 58-year-old Negro woman, was first seen in the General Outpatient Department and directed to the Ear, Nose, and Throat Clinic; there, a tentative diagnosis of allergic rhinitis was made. She was placed on oral antihistaminics and nasal vasoconstrictors which were of only transitory benefit. After a trial period of this therapy, she was referred to the Allergy Clinic for further study (June, 1953).

Salient points in the patient's history were as follows: She had been relatively well most of her life. She had been examined two years previously because of complaints of gas, eructation, and pressure in the upper abdomen; however, no organic basis for these symptoms could be demonstrated. On the present occasion, the patient asked for advice because of persistent watery nasal discharge of two months' duration. She reported only occasional and not paroxysmal sneezing. There was occasional but not persistent nasal blockage. Inquiry about itching of the eyes, nose, ears, or palate received only a vague noncommittal reply. The chief symptom, nasal discharge, had a definitive, rather than gradual, onset, coinciding with a change in environment and season.

The patient had no previous personal or family history of allergy. She stated specifically that her symptoms began after moving to a new apartment. Close questioning about the new living quarters indicated that these had been freshly painted prior to her occupancy, and that the furniture was new. She had no pets. The apartment was relatively free from dust, but there was a large empty lot across the street where grasses,

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trees, and weeds were plentiful. The first symptoms had occurred in late April or early May. The rhinorrhea was most marked at night. During the day, symptoms were alleviated when she left the house.

Complete physical examination was not helpful. No definite clue was obtained pointing to allergic etiology. The nasal mucous membranes were pale, only moderately swollen, with several mildly inflamed patches. Skin tests with environmental and seasonal inhalants were negative, or in the case of atmospheric molds, equivocal. The circulating eosinophils were within normal limits. The nasal secretion was free of eosinophils.

An hour-by-hour diary of activities and symptoms failed to provide clues of the nature of specific offenders. Results from a three-week vacation in the South, within the region of the Mississippi Valley, were disappointing, although it was recognized that vegetation and other potentially allergic factors were not markedly different in the vacation area. The nasal discharge continued; there was only slight, if any, change in the nasal blockage or in the tendency to sneeze. Once again, rather vague replies were given to inquiries about watering, redness, or itching of eyes, and other symptoms of allergy. In essence, the change in environment was not associated with any change in type, frequency, or severity of symptoms.

Further attempts with symptomatic treatment remained unsuccessful. After three months of diagnostic and therapeutic effort, the case was reviewed. Requestioning highlighted the relatively profuse nature of the rhinorrhea. In order to ascertain the characteristics of the nasal secretion, a test tube was inserted into the right nostril, and the secretion collected by gravity for thirty minutes. Twenty milliliters of clear fluid was obtained. The lack of cloudiness and profuse flow encouraged laboratory analysis, which disclosed a composition as follows: 29 mg. per cent protein, 123 meq. chloride, and, most importantly, 70 mg. per cent glucose. Nasal secretion is ordinarily free from glucose, but contains mucopolysaccharide, which was absent from this specimen. On the basis of these findings, it appeared likely that the rhinorrhea reflected cerebrospinal fluid leakage, rather than allergic rhinitis.

The patient steadfastly maintained that she had suffered no trauma. Abnormal neurologic signs or symptoms were absent. X-ray revealed no evidence of fracture or neoplasm, nor of abnormality involving the cribriform plate. The X-ray Department's report drew attention to the possible significance of some demineralization of the posterior clinoids, and it was also observed that unusually large paranasal air sinuses were present.

The Department of Neurology agreed that the findings were sufficiently indicative to forego confirmation by intraspinal injection of indigo carmine and subsequent demonstration of the dye in the nasal fluid. The patient was transferred to that Department where she submitted to surgery for repair of the defect which permitted escape of the cerebrospinal fluid.

A craniotomy was performed through a horseshoe right frontal scalp incision, following which several burr holes were made, whereby a bone flap was turned downward, hinged on the right temporal muscle. The anterior superior frontal burr hole entered the large right frontal sinus, which was then treated with Gelfoam at the superior pocket. The right frontal pole was then retracted upward by silver retractors, and the cribriform plate area exposed extradurally. Since the dura was tightly bound down in the region of the cribriform plate, it was entered over the roof of the right orbit, and the incision carried to the falx. Large cottonoid patties were placed on the orbital surface of the frontal pole which was then retracted and freed of cerebrospinal fluid by suction. The olfactory bulb was clearly demonstrated, and lay freely on the place. Beneath the bulb the cribriform plate was found to contain several small holes measuring 0.5 to 2 mm. No olfactory fibers were found in these openings, and it was assumed that this was the site of the leakage of cerebrospinal fluid. These openings were closed by bone wax, over which a strip of temporal fascia was placed, and, in turn, a similar piece of Gelfoam was again superimposed. The frontal pole was then replaced into position, and the dura closed. The bone flap was in turn replaced after hemostasis was obtained with bone wax. Recovery was uneventful, except for mild right lateral episcleral hemorrhage occurring about four weeks postoperatively, which required no

treatment. The patient was discharged three weeks after surgery. Rhinorrhea was absent on discharge, and had not reappeared seven weeks after surgery. The final diagnosis was cerebrospinal rhinorrhea, of unknown origin.

DISCUSSION

Cerebrospinal rhinorrhea may occur from (1) trauma, (2) neoplasm, (3) congenital anomaly, and (4) spontaneously. Cairns[1] names four possible groups: (1) acute head injuries, (2) delayed complication of head injuries, (3) results of nasal operations, (4) spontaneous. Coleman and Troland[2] state that for the truly spontaneous type to occur the following must be absent: trauma, infection of the bones of the paranasal sinuses, tumors eroding the base of the cranium, prolonged increase in cerebrospinal fluid pressure, and congenital anomalies. In short, there must be no demonstrable cause.

The most probable route for escape of cerebrospinal fluid follows the path of the olfactory nerves. The filaments, passing to the bulb, are held in place as they pass through the cribriform plate by extension of the three layers of the meninges. The dura mater joins the periosteum of the nose, the pia mater fuses into the neurolemma of the nerve fibers, while the arachnoid does not usually extend through the cribriform plate. Why a rupture in the arachnoid should occur spontaneously remains unknown. Juaregg[3] refers to the possibility that sneezing may cause increased intracranial pressure, which most investigators consider to be a prerequisite for development of spontaneous cerebrospinal rhinitis.

Although cases of spontaneous rhinorrhea are quite rare, the following have reported cases which appear to have met the criteria listed above: St. Clair Thomson,[4] Johnston,[5] Adson,[6] Friedberg and Galloway,[7] Plum,[8] Fox,[9] Wessels,[10] Ballon and Ballon,[11] Titche,[12] Jobson,[13] Dandy,[14] Wurster,[15] Feinblatt and Damrau,[16] Ameriso,[17] and Coleman and Troland.[2]

SUMMARY

A case of spontaneous cerebrospinal rhinorrhea simulating allergic rhinitis is reported. The apparent relationship between environmental changes and onset of symptoms was responsible for the presumptive diagnosis of allergic rhinitis. Allergic survey was negative. Symptomatic medication failed to bring relief. Chemical studies of the nasal secretion, conducted after therapeutic failure became evident, revealed the similarity of the nasal fluid to cerebrospinal fluid. Consultation with the Department of Neurology confirmed the likelihood of a defect in the cribriform plate, permitting escape of cerebrospinal fluid. Surgical closure terminated the rhinorrhea.

I wish to express appreciation for the generous help and encouragement given by Dr. Max Samter, Head of the Allergy Clinic, and to Dr. Eric Oldberg, Head of the Departments of Neurology and Neurosurgery, for kind permission to include neurosurgical aspects of the case.

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Editorial

THE ALLERGIST AND PREVENTIVE MEDICINE

AMONG the greatest benefits of modern medicine are the methods of preventing disease and of detecting serious illness in its earliest and most curable stage. Many large corporations find it profitable to protect their interest in highly trained and experienced executives by subjecting them annually to elaborate physical and laboratory examinations. When the initiative and expense are left to the individual, the periodic health examination is likely to be neglected. Most parents start their infants in life under careful medical supervision and with a full quota of immunizations, but as the child grows older examinations are less frequent and, after the end of parental control, contact with the physician is usually limited to times of dire disability. Many an adult who conscientiously sees his dentist twice a year either has no family doctor or hesitates to consult him unless he feels sure the findings will amply justify the visit.

While preventive medicine is logically a part of general practice, all doctors should aid in giving their patients the benefits of it. Among the specialists, the allergist has an unusual opportunity, since a large proportion of his patients, by the nature of their illness and treatment, must make periodic visits over long periods of time. Aside from the allergic diseases which are their reason for coming, they represent a cross section of the population needing preventive medical advice and, in many cases, their visits to this office constitute their principal contact with the medical profession.

The possibility of preventive care will depend greatly on the nature of the contact with the patient. The initial history and physical examination must be sufficiently complete to indicate an interest in the patient as a whole, rather than merely the manifestations of allergic disease. Then the patient will not hesitate on later visits to ask advice on matters of health and symptoms for which he would hesitate to make a visit to another doctor, and which might be neglected if the allergist were thought to be concerned only with his narrow field.

Our purpose is to remind the allergist of this opportunity, rather than to outline a specific program, which will obviously vary in different cases. However, a few simple examples may illustrate the type of procedures desirable. It is important to learn not only if the patient has a family doctor, but

whether he sees the doctor often enough to allow reasonable preventive medicine. Have a blood count, sedimentation rate, routine urinalysis, and chest x-ray examination been done lately? Has a young patient received diphtheria and tetanus toxoids, pertussis and smallpox vaccines? Has the elderly patient had an electrocardiogram? When skin tests are done they may well include horse serum, tuberculin, and Schick toxin, as well as those pertinent to the present complaint. During treatment, the weight and blood pressure may be followed periodically, together with any other observations suggested by earlier studies.

The allergist need not fear that he will become a general practitioner or compete with the colleagues who refer patients to him. His role is simply to warn the patient of danger and see that he receives care from his family doctor or an appropriate specialist, a service for which both doctor and patient will be grateful.

American Academy of Allergy

THE AMERICAN ACADEMY OF ALLERGY POSTGRADUATE TEACHING PROGRAM

FRIDAY, SATURDAY, AND SUNDAY, FEB. 4, 5, AND 6, 1955, NEW YORK, N. Y.

Contact Dermatis — Friday, Feb. 4, 1955
Roosevelt Hospital, 428 West 58th St., New York City
School of Nursing Auditorium
9:30 A.M. to 12 Noon—2 P.M. to 5 P.M.

The subject of contact dermatitis will be presented from a practical, clinical point of view. It will include case presentations with differential diagnosis, techniques in diagnosis, and treatment, both symptomatic and immunological. The clinical problems of skin sensitization to a wide variety of chemicals and drugs, poison ivy, and industrial dermatoses will be emphasized.

Asthma — Saturday, Feb. 5, 1955
The New York Hospital, 1300 York Ave., New York City
Main Auditorium B-011
9:30 A.M. to 12 Noon—2 P.M. to 5 P.M.

The session on asthma will be devoted to practical clinical case presentations from various of the New York and Brooklyn allergy clinics. The cases will be both adult and pediatric and will be selected to demonstrate several varieties of clinical asthma, methods of diagnosis and testing, immunization, and newer forms of therapy. The problems of hypersensitivity, infection, and psychological factors will be presented in individual cases.

Panel Discussion — Sunday, Feb. 6, 1955
The Mt. Sinai Hospital, 103rd St. and Fifth Ave., New York City
Blumenthal Auditorium
9:30 A.M. to 12:30 P.M.

The panel will be devoted to a discussion of asthma on the basis of questions arising from the session of Saturday.

There will be no charge or registration fee. For further information, contact the American Academy of Allergy, 208 East Wisconsin Ave., Milwaukee 2, Wisconsin.

Abstracts of Papers to Be Presented at the Eleventh Annual Meeting of the American Academy of Allergy

1. A CONTROLLED STUDY ON THE USE OF PARENTERAL AND ORAL ANTIHISTAMINES IN PREVENTING PENICILLIN REACTIONS

KENNETH P. MATHEWS, M.D., FAY M. HEMPHILL, PH.D., JOHN M. SHELDON, M.D., ROBERT G. LOVELL, M.D., AND WARREN E. FORSYTHE, M.D., SC.D.

Reports indicating that the simultaneous administration of antihistamines along with penicillin significantly reduces delayed, as well as immediate, reactions to the latter drug require confirmation in view of the obvious practical importance of such observations. This study is concerned with more than 2,000 courses of penicillin given to students at the University of Michigan Health Service during the past two years. Before receiving the drug, all patients filled out a form giving pertinent information regarding previous courses of penicillin, personal and family history of allergy, fungus infections, etc. In rotation, one-half the patients were given ordinary fortified procaine penicillin and one-half were given the same drug diluted with a solution containing 10 mg. of chlorprophenpyridamine (Chlor-Trimeton). One-half the patients in each group were given a supply of placebo tablets and one-half were given oral Chlor-Trimeton, thus making a total of four groups. The tablets were prescribed for the duration of penicillin therapy and ten days thereafter. The patients were followed for at least three weeks, written forms being submitted after this length of time indicating how regularly the tablets were taken, their side effects, etc. Possible reactions were evaluated by a single physician without knowledge as to the type of penicillin or tablets received by the patient. The data were punched on IBM cards for analysis.

No significant difference in the incidence of severe or delayed penicillin reactions was noted between those receiving oral or parenteral antihistamines and the controls.

2. STUDIES IN PENICILLIN HYPERSENSITIVITY. I. IMMUNOLOGIC FINDINGS IN TWO PENICILLIN SENSITIVE PATIENTS.

BERNARD B. SIEGEL, M.D., MONROE COLEMAN, M.D., LESTER WEISSMAN, M.D., AND MERLE SHER, M.D.

Two nurses with marked sensitivity to penicillin were observed for approximately two years. Both had experienced severe immediate allergic reactions to penicillin, administered orally in one case and intramuscularly in the other. Both patients also experienced near-fatal constitutional reactions to intracutaneous tests with the antibiotic.

Blood specimens were drawn from these two patients at varying intervals during the period of observation and the sera were titered for penicillin reagins by the serum dilution technique.

The persistence of reagins in the blood stream varied in the two patients. One showed a rapid decline within several weeks, while a slowly decreasing titer has been noted for more than a year in the other. Despite relatively low reagin titers with both sera, strong contralateral passive transfer reactions (Fernauslösung) were obtained, even with diluted serum.

Antigen dilution studies done on sites passively sensitized with full-strength serum revealed positive reactions with a test dose of penicillin containing .000005 unit. Other immunologic studies will be reported.

3. NASAL HYDROCORTISONE IN ALLERGIC RHINITIS: A CONTROLLED STUDY

HENRY D. OGDEN, M.D., HULDAH BANCROFT, PH.D., JACK RULI, M.D., WILLIAM STEPHENS, M.D., AND CECIL BRANSON, M.D.

This is a study of the value of hydrocortisone acetate (15 mg. per cubic centimeter) in the treatment of allergic rhinitis. Patients selected for study come from the outpatient

allergy clinics of the Charity Hospital of Louisiana. All have complaints suggestive of allergic rhinitis. The patients are divided into three groups. One group is receiving the hydrocortisone, one group a placebo control, and the third is being alternated every three weeks, half being on hydrocortisone while the other half is on placebo.

Chest plates and electrocardiograms were taken on each patient at the beginning of the study. The patient's weight is recorded weekly and periodic urinalyses and blood pressure determinations are being done.

Appraisal is made by both subjective and objective measures. Patients are seen weekly, reporting on the amount of sneezing, nasal blockage, phlegm in the throat, etc. At the same time they are seen by the ear, nose, and throat department who record color of nasal mucosa, turbinate size, degree of bogginess, polyps, and postnasal drip. Nasal smears are examined every three weeks.

The study is wholly blind in that the placebo and hydrocortisone are labeled only A and B and those doing the work do not know which is which. In addition, the ENT men who are responsible for the objective appraisal do not know to which group the patient they are examining belongs.

4. CORTISONE OR HYDROCORTISONE IN THE TREATMENT OF SEVERE, INTRACTABLE BRONCHIAL ASTHMA?

WALTER S. BURRAGE, M.D., DAVID M. K. WANG, M.D., AND JOHN W. IRWIN, M.D.

Recent reports have appeared comparing the use of cortisone and hydrocortisone in the treatment of bronchial asthma. Authors do not agree as to which hormone is more effective.

From April, 1951, to May, 1953, twenty-two patients with severe intractable asthma of unknown etiology were cleared and maintained symptom-free with cortisone. The optimal daily dose was determined by lowering the daily dose in steplike fashion until symptoms returned. The daily dose was then placed just above the amount which failed to control symptoms. Repeated attempts to lower the maintenance dose were made. Starting in September, 1953, nine of the patients on maintenance cortisone were switched to the same amount of daily hydrocortisone. This dose was lowered until symptoms of asthma began to appear. Again, the maintenance level was placed just above the level at which symptoms started to reappear. The other thirteen patients remained on daily cortisone. In January, 1954, eight patients with similar asthma were placed on hydrocortisone. After symptoms cleared, the optimal level of daily hydrocortisone was found for each patient.

A statistical analysis of these doses will be presented. It will be possible to compare total clearing amounts and optimal daily maintenance doses of both cortisone and hydrocortisone with some degree of certainty. Complications of maintenance cortisone and hydrocortisone will be compared.

5. CORTICOTROPHIN, CORTISONE, AND HYDROCORTONE THERAPY IN ASTHMA IN RELATION TO HYPERGLYCEMIA AND DIABETES MELLITUS

B. ROSE, M.D.

During the course of hormone therapy for asthma, the possibility of inducing permanent diabetes or other metabolic changes has always been considered. With this in mind, routine carbohydrate tolerance curves, as well as other determinations such as serum cholesterol, have been made in patients receiving hormone therapy for long periods of time. Out of a group of some 400 patients, many of whom have received treatment for over three years and a few since 1949, four cases of diabetes have developed. These findings will be presented in relation to steroid metabolism and the possible relation of asthma to diabetes mellitus.

6. PSYCHIATRIC, PULMONARY, AND ADRENOCORTICAL OBSERVATIONS IN BRONCHIAL ASTHMA

PETER H. KNAPP, M.D., AND ALAN MICHELSON, M.D.

In a coordinated psychiatric, clinical, and biochemical study of bronchial asthma, we have compared the results of intensive psychiatric and psychologic interviewng with those obtained by careful clinical rating by a score sheet method and by examination of the urinary steroid output.

Examination of a large population of asthmatic subjects showed a wide range of personality disturbance. Psychiatric estimates of the severity of disturbance correlated significantly with independent estimates by allergists as to the severity of the pulmonary disability. We have found a reduction in steroid output among subjects having asthma, compared with a control group. Asthmatic patients, furthermore, show a marked fluctuation in the level of steroid excretion measured at weekly intervals. It appears to fall following exacerbations of their illness, whereas at the onset of their illness there are often sudden rises in output which occur at times of turmoil and widespread personality disturbance.

Case material is offered to show our method of combined approach and to illustrate these findings.

7. STUDIES ON THE SOURCE OF THE HOUSE DUST ANTIGEN

EDNA M. FOLLENSBY, M.D., FRANCIS C. LOWELL, M.D., AND IRVING W. SCHILLER, M.D.

Antisera prepared in rabbits injected with a house dust extract (antigen A) incorporated in an adjuvant were tested by the interfacial, complement fixation, and agar diffusion techniques, with antigen A, with a purified fraction obtained from house dust, (antigen B), and against antigens prepared from those bacteria which we have isolated so far from old cotton mattress stuffing and a sample of house dust (antigens C). The reactivity of these antigens was also determined in the skin of normal and dust-sensitive persons.

The following conclusions are based on results obtained to date: antigen A fixes complement well and contains at least two distinct antigenic components; antigen B, which reacts feebly by precipitation, less well than antigen A by complement fixation, and is distinctly less reactive in the skin of dust-sensitive persons, is either antigenically different from antigen A or possesses the same or similar antigens in lesser concentration. We are inclined to the latter view. Antigens C are questionably reactive by precipitation, fix complement irregularly and only in high concentration, and do not appear to be significantly reactive on the skin of normal and dust-sensitive persons.

8. THE SIGNIFICANCE OF THICKENED BASEMENT MEMBRANE IN THE BRONCHIAL TREE

S. B. CREPEA, M.D.

The usual pathology of asthma is such that a thickened basement membrane might be an important permanent change and the only evidence of a pre-existing asthma. Sections of lungs taken from routine autopsies and from the surgical pathology section were reviewed for the incidence of thickening of the basement membranes in nonallergic persons. Thickening was found to correlate with the age of the patients and with chronic pulmonary disease. It was not as marked or constant a finding as in asthma, but it was frequent enough and advanced enough in nonallergic people to be of no diagnostic significance by itself. Photomicrographs and statistics will be presented.

9. SINUS DISEASE, BACTERIAL ALLERGY AND BRONCHIAL ASTHMA.

S. SIEGAL, M.D., J. L. GOLDMAN, M.D., L. ARNOLD, M.D., AND C. HERSCHBERGER, B.S.

Seventy-five cases of intrinsic asthma and thirty-two cases of allergic asthma were studied clinically, radiologically, and bacteriologically as to paranasal sinus status. Sixteen intrinsic cases had a special study of sinus membrane micropathology and bacteriology.

Asthmatic responses to multiple provocative injections of sinus vaccines were noted in a small series. Twenty-nine cases of intrinsic asthma had a follow-up observation two to fourteen years after bilateral radical sinus surgery.

Active sinus infection was the exception in both intrinsic and allergic asthma. In thirteen cases of intrinsic asthma there was neither sinus membrane alteration on x-ray nor bacteriologic evidence of sinus infection. However, the greater incidence of more extensive sinus membrane change and of sinus infection in intrinsic asthma is confirmed. In sixteen cases of intrinsic asthma, bacterial stain of thickened sinus membrane did not reveal microorganisms in a single instance. Most membranes were found uninfected, and seven with the typical pathology of edema and eosinophils yielded no growth or nonpathogens only. Multiple provocative tests with sinus vaccines failed to induce consistent asthmatic responses. Radical sinus surgery follow-up showed few cases with significant benefit, a preponderance of cases with regrowth of naso-sinal polypoid membrane, and a poor correlation between result as to asthma and result as to sinus status. The findings of this study fail to support the hypothesis that intrinsic bronchial asthma or its associated sinus membrane disease are due to bacterial allergy.

10. IODIDE SENSITIVITY IN THE ETIOLOGY OF PERIARTERITIS NODOSA

HOWARD RASMUSSEN, M.D.

Hypersensitivity as an etiological agent in periarteritis nodosa was first suggested by Gruber. This concept was further amplified by Rich. The latter, in the course of his studies, reported one case in which iodides were implicated as the etiological factor.

In spite of the widespread use of iodine compounds in various x-ray procedures and in the treatment of thyroid and pulmonary diseases, there has been only one subsequent report suggesting an etiological role of these compounds in cases of periarteritis, although less severe sensitivity reactions have been described frequently.

In the past three years, in Massachusetts General Hospital, two cases of periarteritis have been seen in which iodides seemed to have played a role. These cases were well studied clinically and pathologically. Both patients were women, whose initial illnesses were diagnosed as uncomplicated bronchial asthma. The one had had bronchial asthma for over fifteen years, and during this time had received potassium iodide on at least one occasion. One week after the reinstitution of potassium iodide therapy, she developed a severe diffuse vasculitis. After a five months' hospitalization she had a remission, and except for continued asthma, is living and well two years later. The other had had asthma for one year, and during this time had taken potassium iodide on two occasions. On each of these occasions she developed urticaria which promptly subsided with cessation of therapy. She received potassium iodide a third time and twenty-four hours later developed symptoms. She continued the drug for ten days with progressively more severe symptoms. The potassium iodide was stopped and she was given a short course of cortisone, with prompt subsidence of all symptoms. With cessation of cortisone therapy, she had a marked recrudescence of symptoms and signs and died three weeks later. Serum from this patient gave a positive PK reaction to potassium iodide in two of three persons, and a positive reaction to aspirin in one of three persons so tested. (She was subsequently given large doses of aspirin without obvious change in her course.)

Since asthma is a common manifestation of periarteritis, it is impossible to prove that these patients did not have the disease prior to iodide therapy, but in neither case was there clinical data to suggest this possibility. In addition, as with most patients with bronchial asthma, they received other agents, including antibiotics, any of which may have played a role.

11. AN EVALUATION OF MOLDS AS AN ALLERGENIC FACTOR IN THE NEW YORK METROPOLITAN AREA. A SEVEN-YEAR STUDY

DAVID MERKSAMER, M.D., AND HYMAN SHERMAN, M.D.

Prior to 1948, the lack of a standard technique for counting atmospheric spores made it difficult to study the importance of molds as allergens. The Research Council of the

American Academy of Allergy adopted the same technique for daily mold counts which it had standardized for pollen counting in 1946. Since 1948, daily mold counts have been made at the Allergy Laboratory of the Jewish Hospital of Brooklyn by one of the authors (D. M.), from April to November and, in some instances, throughout the year. Counts were made at seven to eight stations in the New York metropolitan area for from one to seven seasons. These daily counts were of great assistance in establishing the importance of mold sensitivity as a cause of seasonal allergic rhinitis and bronchial asthma in the New York area. The seasonal totals for seven years and an average seasonal graph will be presented.

A detailed analysis was made of fifty cases which were proved definitely to be mold-sensitive, clinically. Among the many interesting findings were the following: (1) the importance of mold sensitivity in the younger age groups; (2) the high incidence of bronchial asthma; (3) the low incidence of ophthalmic symptoms, despite the presence of positive ophthalmic reactions to testing; (4) the high coincidence of mold and pollen sensitivity, necessitating a careful differential diagnosis for proper therapy; (5) the high incidence of positive passive transfer reactions; and (6) the relatively low tolerance for dosage, resulting in a high incidence of constitutional reactions, which, in children, was in contrast to their good tolerance for pollen therapy.

12. A FIBROCRYSTALLOID ANTIGEN FROM ALTERNARIA

L. A. Sternberger, M.D., A. R. Feinberg, M.D., S. M. Feinberg, M.D., C. Benaim, M.D., and S. A. Warren, M.D.

A dialyzable and a nondialyzable principle were obtained from alternaria grown on a synthetic medium. A fibrocrystalloid antigen was isolated from the nondialyzable fraction by low temperature centrifugal ultrafiltration, deproteinization, and precipitation as the uranyl salt. Fractionation was controlled by skin testing, gel diffusion, and quantitative precipitin analysis. The dialyzable principle is a hapten of competitive specificity to the nondialyzable antigen.

13. THE HISTOLOGIC AND HISTOCHEMICAL CHANGES IN ATOPIC DERMATITIS. I

Ben Z. Rappaport, M.D.

Skin biopsies from patients with atopic dermatitis were prepared by freeze-drying. They were stained by routine methods for histologic study, and by the Hotchkiss-McMannus method for study of the ground substance. These histologic studies indicate that the changes in the mucoproteins during the active phase of the eruption are similar to those found in the nasal mucosa of active nasal allergy. The ground substance stains poorly; the subepithelial basement membrane is thinned; the perivascular basement membrane is thinned. The cytoplasm of the fibroblasts and of the histiocytes stains lightly. These changes indicate a state of depolymerization of ground substance and basement membranes, as well as a decreased concentration of the precursor of mucoprotein in the fibroblasts and histiocytes. The changes will be demonstrated by means of lantern slides.

13A. THE HISTOLOGIC AND HISTOCHEMICAL CHANGES IN ATOPIC DERMATITIS FOLLOWING CORTICOID THERAPY. II.

Ben Z. Rappaport, M.D.

The effects of corticoid therapy on atopic dermatitis will be demonstrated by means of lantern slides. The changes can be characterized as a reversal of the allergic state, a state resembling normal tissue insofar as the mucoproteins are concerned. These changes are the more significant, since nothing except reduction in edema is demonstrable by usual histologic techniques. With the mucoprotein stains (Hotchkiss-McMannus) one observes a deepening in stainability and integrity of the basement membranes and an increase in granule content of the cytoplasm of fibroblasts and histiocytes. The changes are interpreted as being due to a reversal of depolymerization mucoproteins.

14. THE DURATION OF DERMAL SENSITIZATION FOLLOWING CELLULAR TRANSFER IN GUINEA PIGS

M. W. Chase, Ph.D., and J. R. Battisto, Ph.D.

Analysis is made of the persistence of the sensitized state that arises in normal guinea pigs through injection of white cells donated by hypersensitive guinea pigs. The donors were sensitized chiefly by a special two-step method (*Internat. Arch. Allergy* **5**: 163, 1954) so as to give intense contact reactions with picryl chloride. Cells from peritoneal exudates, lymph nodes, or spleens were injected into new animals intraperitoneally or intravenously in amounts of 0.07 to 0.9 ml. The recipients were tested at selected intervals up to 100 days by contact tests and at times also by intradermal injection of conjugated "picrylated guinea pig serum" or of picryl chloride.

Sensitivity arose in from nine to twenty-two hours after injection of the cells. Daily contact tests revealed a characteristic diminution in response by the sixth or seventh day; thereafter, animals that had acquired a moderate degree of sensitivity usually lost it, while those originally highly sensitive often regained their initial sensitivity and retained it undiminished for many weeks. The persistence of sensitivity apparently is influenced by the very contact tests used for ascertaining its presence, for sensitivity appears to fall off when the initial testing of recipients (prepared in quadruplicate and tested at various times) is withheld for two or three weeks. When one uses as recipients animals that are specifically resistant to active sensitization because of prior feedings of picryl chloride (*Proc. Soc. Exper. Biol. & Med.* **61**: 257, 1946), the cell-induced sensitivity occurs normally in its initial phase but declines gradually and is lost after approximately four weeks. This finding suggests again that instances of persisting sensitivity may be referable to a secondary phase of active sensitization.

15. SENSITIZATION OF THE HUMAN SKIN: A STUDY OF LOCATION OF SITES AND EXPOSURE TIME OF EXCITANT

Max Grolnich, M.D.

This is a further study into the problem of factors involved in the mechanism of sensitization of the human skin. In establishing techniques of sensitization, such variations in procedure were recognized, as (a) short and (b) prolonged patch test exposures, (c) repetition of patch tests, (d) their total number, and (e) the use of various areas of the body. These factors were considered in order to evaluate the importance of both duration of exposure of the excitant and the location of the treated skin sites. A chemical substance was employed, heretofore not previously used on the human skin, which was found in preliminary observations to be able to sensitize the skin by percutaneous application.

Four variations in procedure were established. These were based on findings in previous experiments and studies in skin sensitization by the writer and others. A fifth procedure, employed for purpose of comparison, was that which is suggested by the U. S. Food and Drug Administration for studying the sensitizing potentials of unknown chemicals. A description of technique and procedures will be given in the paper.

There were 121 subjects, predominantly young adults between the ages of 18 and 40 years. Approximately equal numbers of subjects were treated by each of the five procedures (A, B, C, D, and E). Each person was questioned as to personal and family history of atopy, and the occurrence of poison ivy or other contact dermatitis in the past.

Findings.—The percentages of subjects sensitized by groups were: A, 19 per cent; B, 4 per cent; C, 32 per cent; D, 10 per cent; and E, 4 per cent.

The writer will try to interpret the findings in terms of previous experimental studies.

16. ARTHUS DESENSITIZATION

Oscar Swineford, Jr., M.D., and Louise Tull, B.A.

Rabbits have been immunized with egg albumin, ascaris polysaccharide, and BCG vaccine. Intradermal skin tests with strong antigen solution have elicited strong delayed violaceous reactions.

Antigen is given subcutaneously, then intravenously, until the precipitin titer is markedly reduced. The delayed skin reactions become negative or equivocal.

The precipitin titers and skin reactions recover their former intensities in two or three days. However, if the intravenous antigen injection is continued, the suppression of the precipitin titers and the skin reactions can be maintained for a week or so, but not indefinitely.

So far as we know, no one has attempted to study the duration of arthus desensitization by continued injections of antigen.

17. CORRELATION OF BLOOD HISTAMINE RELEASE AND SKIN TEST RESPONSE TO MULTIPLE ANTIGENS

JOSEPH W. NOAH, M.D., AND ALTA BRAND

The technique of blood histamine release, as previously described (J. ALLERGY **25**: 210, 1954), has been employed heretofore only with the blood of ragweed-sensitive persons, using ragweed antigen as the histamine-releasing agent. This study consists of an extension of the basic technique by employing other antigenic extracts to release histamine into the plasma. Individuals were selected as donors because of the severity or variety of their allergic sensitiveness and, in some cases, because of poor or absent skin test responses to conventional intradermal testing.

From each individual sufficient blood was obtained to permit incubation of aliquots with extracts of five common inhalants and six common foods. At the time blood was obtained, intradermal skin tests using the same extracts were carried out and the results were recorded photographically. Histamine liberated into the blood plasma was determined by the method of Graham and Lowry, and the results of these procedures were compared with the results of skin tests performed.

Although, in general, there was a degree of correlation between the skin test and histamine release results, there were surprising discrepancies. In some individuals showing little or no skin test reaction, good blood histamine release was obtained, correlating well with the clinical story. The variations from anticipated results will be discussed.

18. A NINE-YEAR STUDY OF THE EFFECTIVENESS OF NEW YORK CITY'S RAGWEED EXTERMINATION CAMPAIGN

MATTHEW WALZER, M.D., AND BERNARD SIEGEL, M.D.

This study was made at the Allergy Laboratory of The Jewish Hospital of Brooklyn. The technique for pollen counting standardized by the Pollen Survey Committee of the American Academy of Allergy was employed. From 1946 through 1954, daily pollen counts were made during one to nine ragweed seasons at thirty-two different stations in the New York Metropolitan Area. In all, ninety-nine seasonal surveys were completed. The daily counts were correlated with the meteorologic data obtained from weather stations in this area.

Starting in 1946 and continuing to date, the New York City Department of Health has carried on a campaign aimed at the eradication of ragweed plants within the city limits. Each year roadside ragweed plants in all boroughs were sprayed with 2-4D either preceding or during the pollinating season. The statistics on the acreage sprayed each year will be presented.

The average seasonal totals for ragweed pollen in New York City during the nine-year period will be reviewed. Data for representative stations in various city boroughs will be compared with those of stations outside the city limits where there were no attempts at ragweed extermination.

The data accumulated during the nine-year survey have yielded concrete evidence as to the limited effectiveness of extermination campaigns which are carried on purely as local projects. The importance of wind as a factor in preventing the success of such campaigns will be stressed. The possible benefits and limitations of such campaigns will be discussed.

19. STUDIES ON AEROALLERGENS

CLIFFORD GURNEY, M.D., AND SYDENHAM CRYST, M.D.

Although it has been established that ragweed pollen is liberated in the morning, little work has been done to assay the fluctuations in concentration of pollen during the day.

Pollen counting by the conventional gravity method is of limited clinical value because the results are not readily converted to antigenic mass to which patients are exposed. We wish to propose a technique for determining aeroallergen concentrations which may be of greater clinical value, as well as being a useful research tool, and to report the results obtained.

The millipore filter is paper-thin, containing approximately 5 by 10^7 pores per square centimeter. Using such filters and a simple apparatus consisting of a vacuum pump, valves, and a timing mechanism, all particles greater than 0.2 in size are recovered from a known volume of air. With this apparatus, serial determinations at four-hour intervals are being made throughout the ragweed season. The filters are mounted directly on microscopic slides, stained, and counted.

The advantage of this method is that aeroallergens in a known volume of air during a short interval of time may be determined accurately. The method is simple and the results are reproducible, quickly determined, and meaningful. The purpose of this paper is to present a method for aerobiologic surveys, to compare the results so obtained with the "gravity" method, and to demonstrate the clinical relationship between patients' symptoms and antigenic mass. The study was begun Aug. 1, 1954, and will be reported.

20. PRELIMINARY OBSERVATIONS ON THE CHEMICAL NATURE OF ASTHMATIC SPUTUM

SAMUEL C. BUKANTZ, M.D., AND ALINE W. SCHIELE, A.B.

There has been considerable recent interest in the therapeutic use of enzyme or detergent aerosols for the lysis of the thick, viscid mucus which characteristically accumulates intrabronchially in intrinsic asthma. Although the widespread accumulation of such mucus has long been recognized as a characteristic pathologic finding in asthma, relatively few investigations of the chemical nature of the mucus have been recorded. The identification and possible isolation of the specific chemical structure or structures responsible for mucus viscosity would seem to be essential, both to the analysis of the mechanism by which the intrabronchial accumulation occurs and to the selection of the best possible mucolytic agents for therapy.

The first phase of the present investigation has been concerned with the chemical analysis of twenty viscous sputa obtained from asthmatic patients since the winter of 1953. After dilution with distilled water, the sputa were precipitated at 65 per cent final ethanol concentration and the precipitates were dried and analyzed. Total sputum solids ranged from 13 to 33 mg. per milliliter, while the 65 per cent ethanol insoluble fractions ranged from 6 to 19.1 mg. per milliliter and contained carbohydrate, 6 to 28.2 per cent; hexosamine, 12 to 15 per cent; and nitrogen, 9 to 13 per cent. Chromatographic analysis of the various fractions yielded no evidence of distinctive differences among the various sputa. Current experiments involve the use of pepsin digestion and phenol extraction of whole sputum and the insoluble precipitates in the effort to isolate and characterize a mucopolysaccharide.

In one experiment with paper electrophoresis, a somewhat viscous phenolsoluble extract of sputum moved inhomogeneously but in the opposite direction from that taken by pure blood group A and B substance on the same paper strip. Further studies are in progress to ascertain whether blood group substance is present in asthmatic sputum in significant amounts and whether it may contribute appreciably to viscosity.

21. THE SERUM ELECTROPHORETIC PATTERN IN EXPERIMENTAL HYPERSENSITIVITY IN RABBITS

LEO H. CRIEP, M.D., A. I. SCHEPARTZ, PH.D., AND MACY LEVINE, M.D.

In order to study further the role of immunologic factors in experimental vascular hypersensitivity, the present investigation of serum electrophoretic patterns in sensitized rabbits was undertaken.

One series of fourteen rabbits was given a single intravenous dose of bovine plasma albumin in a dosage of 0.25 Gm. per kilogram of body weight. Blood samples were obtained just prior to sensitization and again just prior to sacrifice in two weeks. Histologic studies

showed a high incidence of cardiovascular and renal lesions resembling those seen in periarteritis nodosa in man. Comparison of electrophoretic patterns of sera obtained at two weeks with initial sera showed a significant rise in γ-globulin and α-globulin and a decrease in albumin.

In a second series of fifteen rabbits sensitized in the same manner with bovine plasma albumin, treatment with cortisone, 10 mg. daily intramuscularly, was started immediately following the sensitizing injection. Blood samples were obtained prior to sensitization and again prior to sacrifice in two weeks. Preliminary observations on the electrophoretic patterns suggest a response differing from the initial experiment.

Quantitative antibody studies were done on all sera following sensitization. The findings were compared with the extent of vascular changes and the electrophoretic patterns. The quantitative antibody figures seem to run parallel to the extent of vascular damage.

22. FURTHER STUDIES ON THE FRACTIONATION OF RAGWEED POLLEN EXTRACTS

ELY PERLMAN, M.D.

Previously reported studies have shown that ragweed pollen extracts contain more than one active constituent. Separation into several distinct and reproducible active components has been accomplished by the author and others by means of paper chromatography and mild solvents. This contribution deals with large-scale fractionation using synthetic ion exchange resins and involving mild procedures with no alteration in pH. Further separation of fractions and characterization of these fractions were provided by paper electrophoresis.

23. ELECTROPHORETIC SEPARATION OF SKIN-SENSITIZING ANTIBODY FROM RAGWEED-SENSITIVE PATIENTS

B. ROSE, M.D., T. W. FYLES, M.D., AND A. A. SEHON, PH.D.

The separation of ten sera from ragweed-sensitive patients into their electrophoretically separable protein components was accomplished by starch electrophoresis. The resulting sera fractions were tested by the passive transfer technique, using nonatopic subjects. It was found that the albumin and alpha-globulin fractions were devoid of any reaginic activity, while both the beta- and gamma-globulin fractions were high in skin-sensitizing antibody.

24. THE EFFECT OF ADRENALECTOMY IN THE GUINEA PIG ON ASTHMA FROM ANTIGEN AEROSOL AND ON ITS INHIBITION BY NONSPECIFIC PROCEDURES

C. BENAIM, M.D., S. M. FEINBERG, M.D., S. A. WARREN, M.D., AND L. A. STERNBERGER, M.D.

We have shown previously that certain nonspecific procedures, such as abscess formation, injection of sodium nucleate, and others, increase the tolerance of guinea pigs to asthma-producing aerosols from antigens. The importance of identifying the mechanism involved necessitates a number of approaches, among which are experiments designed to ascertain whether this action is mediated through the adrenals. This paper will discuss the results of bilateral adrenalectomy in guinea pigs in its influence on the effect of nonspecific procedures in the inhibition of asthma from an antigen aerosol. It also will describe the effects of adrenalectomy on the sensitivity of the guinea pig to the antigen and histamine aerosols.

25. OBSERVATIONS ON THE INTRAVENOUS INJECTION OF ALLERGENIC EXTRACTS IN PATIENTS WITH HAY FEVER AND ASTHMA

FRANCIS C. LOWELL, M.D., WILLIAM FRANKLIN, M.D., IRVING W. SCHILLER, M.D., AND EDNA M. FOLLENSBY, M.D.

In the course of a therapeutic trial as to the effectiveness and practicability of giving intravenous injections of allergens to patients with hay fever and asthma, a number of observations were made on the induced systemic allergic reaction in six patients with nasal or pulmonary allergic symptoms who reacted by skin test to common air-borne allergens.

Five of the patients gave a history of nasal symptoms (hay fever) and one of these also had symptoms suggestive of mild bronchial asthma. The sixth patient had bronchial

asthma and was receiving cortisone, with complete relief of symptoms. None had been currently receiving therapeutic injections of allergenic extracts. In some or all of the patients the following observations were made before and after the intravenous administration of ragweed pollen extract (four patients), timothy pollen extract (one patient), and house dust extract (one patient): (1) titration of skin sensitivity by the dilution technique; (2) titration of skin-sensitizing antibody by both the dilution and neuralization techniques; (3) eosinophil and white cell counts; determination of (4) the amount of complement as determined by 50 per cent hemolysis with the spectrophotometer; and (5) the clotting time.

The patients were observed closely during the procedure, and pulse and blood pressure were determined at brief intervals throughout.

Although many of the observations made were anticipated, the interval between the injection of the allergen and the onset of symptoms was unexpectedly long. Also unexpected was lysis of the formed clot in three blood specimens obtained after the induction of allergic manifestations.

The findings will be presented and briefly discussed.

26. GAMMA-GLOBULIN AND THE ASTHMATIC CHILD

RALPH BOWEN, M.D.

Gamma-globulin as a therapeutic adjunct was appraised in seventy-five cases of asthmatic children with a complete control group. This review represents several years' study of asthmatic children who had previously had diet restrictions, followed hyposensitization programs, received numerous antibiotics, and the majority had been subjected to a tonsil- and adenoidectomy before the review was made. In spite of the afore-mentioned, many had missed considerable school and during the winter months some had been hospitalized. Many of these children had been studied from the standpoint of electrophoretic gamma-globulin patterns.

This study demonstrates that gamma-globulin plays a positive and beneficial role by affording the asthmatic child better resistance to respiratory infections as compared with the control group.

Abstracts of Papers to Be Read by Title

27. CLINICAL STUDIES IN ALLERGY WITH SEVERAL NEW DRUGS

CARL E. ARBESMAN, M.D.

The results of clinical trial with the following medications are reported and discussed.

Drug 1.—Plimasin is a tablet prepared by Ciba Pharmaceutical Products, Inc., consisting of 25 mg. Pyribenzamine and 5 mg. Ritalin. (Ritalin is a substance similar to Dexedrine.) Sixty-one patients received this drug, whose purpose is to avoid the side effects of sleepiness and drowsiness which are often obtained with Pyribenzamine. Of this group of sixty-one patients, over one-half of them had the side effect of sleepiness from Pyribenzamine. None of them had this effect with the combination drug.

Drug 2.—Spansules of Aminophyllin were submitted by Smith, Kline & French. Twenty-six patients were given this drug and its efficacy was compared with other medications orally and also with aminophyllin suppositories. The results and side effects could be discussed.

Drug 3.—A tablet containing 25 mg. cortisone and 4 mg. Chlor-Trimeton was submitted by the Schering Corporation. Thirteen patients with intractable allergic rhinitis of a seasonal nature were given this drug. These patients were resistant to other forms of therapy such as hyposensitization and antihistaminics alone. In all probability, the results obtained (that is, all these good results) were due to the cortisone and not to the Chlor-Trimeton in the tablet. There are no controls of patients with just cortisone alone in this series.

Drug 4.—9 Alpha fluorohydrocortisone was submitted by E. R. Squibb and Sons. This very potent steroid, which has been shown by Thorne and his group in Boston to be twenty-five to fifty times more effective than cortisone or hydrocortisone as far as eosinophil response and

electrolyte changes are concerned, was used in eight asthmatic patients who had been on maintenance therapy doses of either cortisone or hydrocortisone. The dosage was small; only 2 to 4 mg. of this substance was given a day. In each instance the clinical results were poor. The patients did not do well at all with this new steroid and had a recurrence of their symptoms, whereas during the control period on the other steroids they got along well. In addition to this, there was marked retention of sodium in these patients, with a consequent marked gain in weight and edema. Chemistry studies were performed on most of these patients, that is, the sodium and potassium levels for several determinations.

Drug 5.—Dapanone, submitted by Sharp & Dohme, is a drug similar in action to ephedrine. It was given to thirty-four patients and the clinical effectiveness and side effects were noted. It has no particular advantage over ephedrine and did seem to cause a lot more jitteriness and excitability than did plain ephedrine.

28. SPONTANEOUS PNEUMOTHORAX COMPLICATING ASTHMA

S. B. Crepea, M.D.

Six cases of spontaneous pneumothorax complicating active asthma were reviewed, as well as the literature. In all our cases an infectious process in the bronchial tree was present. In only one case was the asthma associated with an ingestant (drug) allergy. The rest were of the intrinsic variety.

29. ALLERGY TO CITRIC ACID—CASE REPORT AND CLINICAL STUDY

Lawrence N. Ettelson, M.D., and Louis Tuft, M.D.

A 37-year-old man, an army veteran under treatment for nasal allergy, asthma, and migraine, had frequent outbreaks of ulcerative stomatitis (canker sores). These usually followed the ingestion of several foods, including citrus fruits (especially lemon) as well as grapes and grape products; they occurred also after ingestion of some commercially packaged foods and carbonated beverages. A common ingredient was traced to citric acid present in the commercial foods and in the offending fruits. Application of citric acid crystals to the oral mucous membrane repeatedly reproduced the lesion within a few hours in the patient, but not in other patients used as controls. Results of mucous membrane contact tests with compounds containing citric acid and other organic acids also are reported. Avoidance of foods containing citric acid was followed by relief of symptoms.

30. AEROALLERGINS IN SEATTLE

Lois Frayser, M.D.

Clinically there is no ragweed pollinosis to date in Seattle and standard pollen counts for three years, correlated with field surveys, show only a rare ragweed type pollen from the false ragweed, *Franseria bipinnatifida,* on the shores of Puget Sound. There is no invasion of ragweed such as occurred in the Wenatchee Valley east of the Cascade Mountains and the Willamette Valley of Oregon. Alder counts are the highest in the country and cause most of the tree pollinosis. Counts taken at the Seattle-Tacoma Weather Bureau in 1954 are higher and correlate more closely with clinical symptoms of patients than counts taken in downtown Seattle.

Mold counts by slide and plate methods show molds throughout the year, predominantly Hormodendrum (Cladosporium), Penicillium, and Pullularia with Botrytis and Epicoccum in late summer. In contrast with most of the country, there is very little Alternaria or Stemphyllium to be found either on slides or by culture.

31. CHILDHOOD COMPLICATIONS OF RESPIRATORY ALLERGIES

William Allen Howard, M.D.

In the literature dealing with the problems of allergy there are numerous references to the usual complications of the respiratory allergies, including bronchitis, bronchopneumonia, bronchiectasis, emphysema, atelectasis, pneumothorax, subcutaneous emphysema, and cor

pulmonale. The majority of these conditions arise in conjunction with long-standing asthma, with or without associated nasal allergy, and are more common in adults than in the young allergic individual. In children there are other complications or associated conditions which are more frequent and more important, but whose significance is not fully appreciated. Some of these are common problems in pediatrics, and their successful management depends upon recognition of the role of allergy in their pathogenesis.

The tissue changes in allergy of the respiratory tract are described and the importance of chronic nasal obstruction as a cause of abnormal pulmonary ventilation is stressed. Special emphasis is placed on the role of respiratory allergy in the development of the so-called "adenoid" facies, malocclusions, otitis media, deafness, sinusitis, chest deformities, and retarded physical growth. The need for early recognition and treatment of childhood respiratory allergies is clearly shown.

32. STUDIES ON THE OPTIMUM CONCENTRATION OF GLYCERINE IN THE PREPARATION AND PRESERVATION OF RAGWEED POLLEN EXTRACT

MARY C. JOHNSON, A.B., ALINE W. SCHIELE, A.B., AND STANLEY F. HAMPTON, M.D.

Extracts of low ragweed pollen (20 Gm. per 100 ml. extracting fluid) containing 0, 5, 10, 20, 30, 40, and 50 per cent glycerine by volume were observed after storage for five and twelve months at refrigerator, deep freeze, and room temperatures, as well as heating at 56° C. for forty-five minutes.

Each extract was evaluated in terms of appearance of sediment, changes in concentration of PTA nitrogen and reducing substances, and loss of activity in neutralization of skin-sensitizing antibody.

The higher the concentration of glycerine, the greater was the protective effect against loss of reagin neutralization activity at the various temperatures studied.

There was essentially no change in PTA nitrogen after heating, regardless of the concentration of glycerine. There was, however, an appreciable loss in PTA nitrogen of the stored extracts, most pronounced in those extracts containing smaller concentrations of glycerine.

The loss in bound carbohydrate varied under the different conditions of temperature. There was no change in any extract after heating at 56° C. for forty-five minutes. However, at room temperature, glycerine in the higher concentration showed a protective effect not evident in the lower concentrations.

An important observation was the clarity of extracts of high glycerine content in comparison with the sedimentation in extracts containing either no glycerine or glycerine of low concentration.

Whereas 30 and 40 per cent glycerine afforded significant protection against deterioration, the greatest protection was observed at the 50 per cent concentration.

33. DERMATITIS VENENATA FROM TREE POLLEN OILS—A CLINICAL REPORT

ROBERT G. LOVELL, M.D., KENNETH P. MATHEWS, M.D., AND JOHN M. SHELDON, M.D.

Dermatitis venenata from plant pollen oils, particularly that caused by ragweed, has been reported in numerous articles. The incidence of contact dermatitis from the ether-soluble fraction of tree pollen must be extremely low. Two cases of dermatitis venenata are reported; both of which had their symptoms coincident with the tree pollination period in Michigan. These patients had typical lesions of plant dermatitis on the exposed areas of their skin. They had positive reactions to patch testing to the tree pollen oils and negative patch tests to other plant oils. Negative scratch and intradermal tests were recorded with the protein portion of the pollens. Oral desensitization was tried on both patients. Testing for possible sensitivity to plant pollen oils in dermatitis venenata might well include representatives of tree pollen oils.

34. OBSERVATIONS ON BLOOD VOLUME IN PATIENTS IN STATUS ASTHMATICUS AND DURING A NORMAL PERIOD

ROBERT G. LOVELL, M.D., JOHN M. SHELDON, M.D., AND KENNETH P. MATHEWS, M.D.

The work of Comroe has shown that in patients with severe pulmonary emphysema there is a definite increase in blood volume in addition to other changes, such as increased cardiac output, lowered arterial oxygen saturation, and elevated blood CO_2. The possibility that there might be an alteration in the total blood volume in patients with severe bronchial asthma prompted an investigation of the blood volume in patients during status asthmaticus and again after the asthma was brought under control. The technique of determining the blood volume was that of utilizing radio-iodinated I^{131} human serum albumin (RISA). The material was injected in known concentration, diluted by the patient's blood, and then removed, and the decrease in concentration was determined by use of a well-typed scintillation counter.

Results of four patients studied indicate that there is very little change in blood volume during status asthmaticus, and when a change is observed it does not seem to be in a unilateral direction. The effect of dehydration in one patient was noted when, during status, her hematocrit was 50 per cent. Following control of her asthma and hydration, the hematocrit fell to 40.5 per cent and the blood volume increased by 1,229 ml.

35. METRORRHAGIA, AN UNCOMMON SYMPTOM OF A CONSTITUTIONAL REACTION

ANGELO L. MAIETTA, M.D.

The human uterus can be involved as a shock organ in a constitutional reaction. A case of metrorrhagia, occurring on four different occasions as a component symptom of a constitutional reaction following pollen desensitization injections, is presented.

The significant possibility that such an eventuality due to overzealous desensitizing therapy may occur in a pregnant allergic patient and thus lead to an inevitable abortion or miscarriage is discussed.

The need for conservative antiallergic management in exquisitely sensitive pregnant allergic patients is emphasized and methods to prevent constitutional reactions are discussed.

36. EXPERIENCE WITH AQUA IVY; TREATMENT OF SENSITIVE PATIENTS AND TOXICITY STUDIES ON THE GUINEA PIG

RICHARD E. PASSENGER, M.D., W. C. SPAIN, M.D., AND MARGARET B. STRAUSS, M.S.

Many courses of prophylactic and phylactic treatment are summarized to present further experience with the use of the alum-precipitated pyridine extract of *Rhus toxicodendron* (Aqua Ivy) developed by Strauss and Spain. Follow-up of sensitive patients showed favorable reports in a high percentage of cases in both groups. Although lack of control cases, uncertainty of actual degree of exposure, and the use of other local and systemic medications make dogmatic statements untenable, the authors feel that clinical experience over a number of years confirms the value of the extract, especially when used with proper timing and booster doses at six-week intervals. Necessary precautions to be taken in the presence of any existing dermatitis will be discussed.

The human prophylactic dosage of Aqua Ivy was administered to twenty-four normal white guinea pigs. These animals remained well throughout the test period; they gained weight and their coats remained sleek and luxuriant. Six of these pigs were autopsied and organs were macro- and microscopically examined, with negative findings. Guinea pigs which received this prophylactic series of injections were no more sensitive to patch test with various dilutions of a pyridine ivy extract than were normal untreated animals. This is in contrast to a group of guinea pigs sensitized to poison ivy by the application of 0.02 ml. of a 1-20 dilution of the 10 per cent pyridine ivy upon patch to the denuded skin; the majority of this sensitized group reacted to a 1-20 and 1-50 dilution of pyridine ivy, whereas the normal and the prophylactically treated groups did not react to these dilutions upon patch test. These results will be tabulated for presentation.

Both animal and clinical results would tend to indicate lack of toxicity with Aqua Ivy.

37. ALLERGENICITY OF MODIFIED AND PROCESSED FOODSTUFFS. VII. GELATIN: ANAPHYLACTOGENIC PROPERTIES OF GELATIN AND ITS PRECURSORS

BRET RATNER, M.D., AND LLOYD V. CRAWFORD, M.D.

The studies detailed in this paper demonstrate that gelatin is a nonantigen and that any antigenicity found in its crude precursor (comminuted ossein) was due solely to contamination with bovine blood elements. In no instance did crude or refined gelatin serve as an anaphylactogen. The presence of contaminating blood elements was found in 56 per cent of the experiments with comminuted ossein and in only 6.6 per cent of the experiments with food grade gelatin. The anaphylactogenicity of the blood elements present in the comminuted ossein and crude gelatin mixtures persisted despite the heat and hydrolytic treatments during the process of gelatin preparation. Whatever blood elements resisted this treatment must be regarded as heat-stable in much the same manner as casein and ovomucoid.

In no instance was there any contaminating blood element found in the highly purified gelatin used for intravenous blood replacement.

38. TREATMENT OF POLLEN HAY FEVER AND ASTHMA WITH AEROSOLS OF POLLEN EXTRACTS; INTRODUCTORY OBSERVATIONS

OSCAR SWINEFORD, JR., M.D., AND DAVIS C. LUCAS, M.D.

In 1953 ten cases of fall hay fever and asthma were treated with aerosols of pollen extract. All the patients had hay fever; four had asthma also. Seven were treated by nasal inhalation alone, two were treated by nasal and transoral routes, and one transorally alone.

Four cases were treated by aerosol as their only specific therapy. The other six received aerosol as a supplement to unsuccessful pollen injections. Relief was estimated at 90 per cent in four, 80 per cent in two, 50 per cent in one, and 25 per cent in one.

About ten additional cases were treated during the 1954 ragweed season. It is too soon for an accurate evaluation of these cases, which have not been reported. The impression is that the results are about the same as for 1953.

39. THE ASTHMAGRAM: CASES ILLUSTRATING ITS USE

OSCAR SWINEFORD, JR., M.D., JESSE W. CUMBIA, M.D., KENNETH W. BERGER, M.D., AND WILLIAM P. COLEMAN, M.D.

The chief purpose of this paper is to introduce the term and the concept of the asthmagram. Cases will be presented to illustrate its use in the practical application of the data presented in "Classification of the Causes of Wheezing" in the March issue of the JOURNAL OF ALLERGY.

The asthmagram consists of the tabulation of all data which can be obtained under the headings of allergy, infection, reflex, psychogenic, physical, chronic lung disease, nonspecific irritants, cardiac asthma, and bronchial obstruction as causes of wheezing in any case of asthma.

Our experience has been that less than one-half of our cases of chronic asthma are due to allergy alone, but the statistics will not be a part of the paper.

40. PREVENTION OR AMELIORATION OF REACTIONS TO SPECIFIC AEROSOLS OF ANTIGENS BY PRETREATMENT WITH AEROSOLS OF ANTIHISTAMINES AND SYMPATHOMIMETICS

OSCAR SWINEFORD, JR., M.D., AND ATWELL MOTLEY, M.D.

A preliminary report of the effectiveness of aerosols of Isuprel and Pyribenzamine in the prevention or amelioration of specific reactions to antigen aerosols is in press in the JOURNAL OF ALLERGY. Since then the effectiveness of several antihistamines and sympathomimetic aerosols has been evaluated. There was a wide difference in the effectiveness of several different preparations in the two types of drugs.

41. EVALUATION OF THE SKIN-TESTING METHODS EMPLOYED IN THE DIAGNOSIS OF PENICILLIN ALLERGY

LOUIS TUFT, M.D., AND DONALD GREGORY, B.A.

Increasing incidence of penicillin sensitization and reaction makes early diagnosis desirable. Recommended tests are widely diversified with no standardization of method, concentration, or amount. The present study attempts to evaluate and, if possible, standardize testing methods and to determine possible usefulness in detection of penicillin allergy. Scratch, patch, and intracutaneous tests were done with Bicillin and Wycillin and their vehicles in varied concentrations, care being taken to watch for delayed reactions after the latter. Tests were done on 134 controls (with no history of previous penicillin administration); on 463 patients with positive history, but no known reaction; and finally upon 32 patients with history of penicillin reactions. Blood of positive reactors was studied for reagins. Repeat skin and blood tests were also done upon these patients to follow course of sensitivity. In all patients receiving penicillin, an attempt was made to ascertain personal or family history of allergy and/or fungus sensitization. Results of the study show that patch test or delayed reactions are of no value in diagnosis and intracutaneous tests are most sensitive in concentration of 10,000 to 100,000 units per cubic centimeter. Low incidence of positive reactions and evanescent character of skin sensitivity as indicated by follow-up studies limit usefulness of skin test methods.

42. STUDY OF THE EFFECT OF PANTOTHENIC ACID UPON ALLERGIC REACTIONS

LOUIS TUFT, M.D., JOAN GREGORY, B.S., M.T., AND DONALD GREGORY, B.A.

Recent reported investigations suggested that oral calcium pantothenate reduced eosinophils and improved allergic patients clinically. The present study was undertaken to verify these claims by studying the effect of administration of the drug upon the direct skin test reaction in suitable allergy patients, both after prolonged administration (one to two weeks) and after single doses. The latter also was used to study the effect upon the passive transfer reactions. In both instances, effect upon eosinophils was determined by counts taken before and after the drug administration. Finally, the clinical effect was determined in a blind study conducted upon ragweed hay fever patients during the hay fever season. Three types of capsules labeled A, B, and C (with identity unknown to prescribing physician), all looking exactly alike, were utilized. One contained placebo (lactose), one contained calcium pantothenate, and the last contained an antihistaminic. Capsules were interchanged; records were kept of their clinical effect, but the sealed envelope containing the identity of the agent used was not opened until final evaluation was made.

The results of these studies indicate that calcium pantothenate has a definite effect upon the allergic skin test reaction, both direct and indirect. This effect was compared with that following administration of epinephrine, an antihistaminic, and the corticosteroids upon the direct skin test reaction of the volunteer subjects.

43. NASAL POLYPS AS RELATED TO ALLERGIC RHINITIS AND BRONCHIAL ASTHMA

LEON UNGER, M.D., AND I. N. SANTOS, M.D.

A brief résumé of the literature will be given, as well as a survey of patients with polyps or tendency to polyposis. Allied allergic conditions, nasal smear examinations, and results of treatment, based chiefly on specific measures of avoidance and hyposensitization, will be summarized. Cortisone therapy and the diminishing use of surgical treatment will also be discussed.

Announcements

Annual Meeting of the Canadian Academy of Allergy

The Canadian Academy of Allergy will hold its annual meeting along with the Canadian Medical Association at its conjoint meeting with the British Medical Association and Ontario Medical Associaton at the Royal York Hotel in Toronto during the week beginning June 20, 1955.

Dr. David A. Long of the National Research Council, Mill Hill, London, England, will be the chief guest speaker and his topic will be "The Influence of Nutritional and Hormonal Factors Upon The Immune and Allergic Responses to Infection."

Further details of the program and the final date of the meeting will be given in a later announcement.

Residency in Allergy

Applications are now being received for residency in allergy at the Veterans Hospital in Aspinwall and/or at the Montefiore Hospital and Medical Center, University of Pittsburgh, Pittsburgh, Pennsylvania. Please send inquires to Leo H. Criep, M.D., Bigelow Apartments, Pittsburgh 19, Pennsylvania.

Book Review

Practice of Allergy. By Warren T. Vaughan, M.D., revised by J. Harvey Black, M.D., ed. 3, St. Louis, The C. V. Mosby Company, 1954, 1164 pages. Price, $21.00.

The publication of a third edition is, in itself, ample evidence of the continuing popularity and success of this treatise on allergy. No doubt this acceptance is due in part to the fact that no other book covers so completely the varied fields of knowledge, immunology, physiology, botany, mycology, food technicology, pharmacology, and others, for which the practicing allergist has frequent need, or describes in such detail the practical points of history taking, skin testing, and treatment. Discussions of controversial subjects are unusually complete with care taken to present all important views and full references to the literature.

In the present edition, new sections on pulmonary function studies and treatment of poison ivy dermatitis have been added. A far greater amount of new material has been incorporated into the previous text so skillfully that the reader is not aware of the transition from the writings of the original author to those of the revisor. The third edition is thoroughly modern, yet it retains the pleasing style of Dr. Vaughan.